EAZA Best Practice Guidelines

Antillean Manatee *(Trichechus manatus manatus)*

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EAZA Preamble
Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the "Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria". These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.

Preamble
This is the second EAZA Best Practice Guideline for manatees. While the first section focus on the biology and conservation status of this species in the wild, the second section deals mainly with the husbandry and the management in Zoos. In the first section relevant data concerning the biology of manatees is presented. It is aimed to

provide useful data that is essential in order to understand the needs of manatees, but also how this animal perceive their environment.

In the second section relevant information about management in Zoos is provided. Keeping manatees in European Zoos started in 1934. It was an Amazonian Manatee that arrived at Wroclaw Zoo. However it was not until the 60ties that manatees, in this case Antillean Manatees, arrived from South America in Europe. In the 70ties first births haven been registered (Amsterdam) and manatees begun to be part of the Zoo collection. Especially Zoo Nuremberg, where since 1977 19 manatees (15 are actually alive) pushed with his successful breeding the population in European Zoos. The experience gained in these 50 years showed that manatees can easily be kept and bred in a Zoo environment. However the last decades also demonstrated that "just a pool of water" is not enough to fulfil their needs. Keeping manatees is demanding and the concept of building natural habitats poses extra challenges to Zoo designers. Especially one area, particularly relevant for keeping manatees, experienced fundamental changes over the time in the last decades: the design and development of a proper functioning Life Support System. This Best Practice Guidelines are aimed to present updated data about different techniques that have demonstrated to provide optimal water quality. Feeding routines and food types, but also different methods in providing the food to the animals is being analysed not only from the nutritional point of view but also as an enrichment factor. In summary this Best Practice Guidelines should be used as a reference in order to define best housing conditions for the animals and also to encourage keepers, aquarists and biologists to try and challenge always new tools, procedures and protocols to guarantee the conditions for the wellbeing of their animals.

Finally, the authors would like to thank Marlous Heukels and Lisette van Leeuwen that, as part of their thesis, wrote a first husbandry guideline about manatees in 2008 that provided useful information. Furthermore many other people gave their expertise to this Guideline: Dr. Katrin Baumgartner, Dr. Greg Bossard, Nina Collatz Christensen, Dr. Joseph Gaspard, Dr. Claudia Gili, Agustín López Goya, Peter Haack, Willeke Huizinga, Tim Hüttner, Dr. Alex Lecu, Radosław Ratajszczak, Eric Bairrão Ruivo, Dr. Günter Strauss, Mauro Tambella, Christiane Thiere and many others. Their precious help is acknowledged.

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SECTION A: BIOLOGY AND FIELD DATA

1. BIOLOGY

2.1 TAXONOMY

Order	Sirenia
Family	Trichechidae
Genus	Trichechus
Species	West Indian Manatee *(Trichechus manatus)*
Subspecies	Antillean Manatee *(T. manatus manatus)*
	Florida Manatee *(T. manatus latirostris)*

The West Indian Manatee *(Trichechus manatus)* belongs to the Order Sirenia and the Family Trichechidae. Currently the Species is divided into the Florida *(T. m. latirostris)* and the Antillean *(T. m. manatus)* subspecies.

Common names	Manatee, Sea cow

However, recent mtDNA studies (Garcia-Rodriguez, et al. 1998; Vianna, et al. 2006) found strong geographic differences in haplotype distributions claiming three different clusters described as being deep as the species-level divisions between *T. manatus* and the Amazonian Manatee *T. inunguis*. The three clusters correspond geographically with:
(1) Florida and the Greater Antilles; (2) Western and Southern Gulf of Mexico, Central America, and NW South America west of the Lesser Antilles; and (3) NE South America, east of the Lesser Antilles. Overall the genetic evidence suggests four clusters of *Trichechus* in the New World one inhabit the Amazon, whereas the other three inhabit coastal areas (Cantanhede, et al. 2005). It has been suggested that the three coastal clusters appear to be as different from one another as they are from the Amazon cluster. It remains an open question whether there are four species, two or a single species with four divisions. This information is quite crucial not only for a better understanding of the evolution of the manatees but also for conservation and In-Situ/Ex-Situ management.

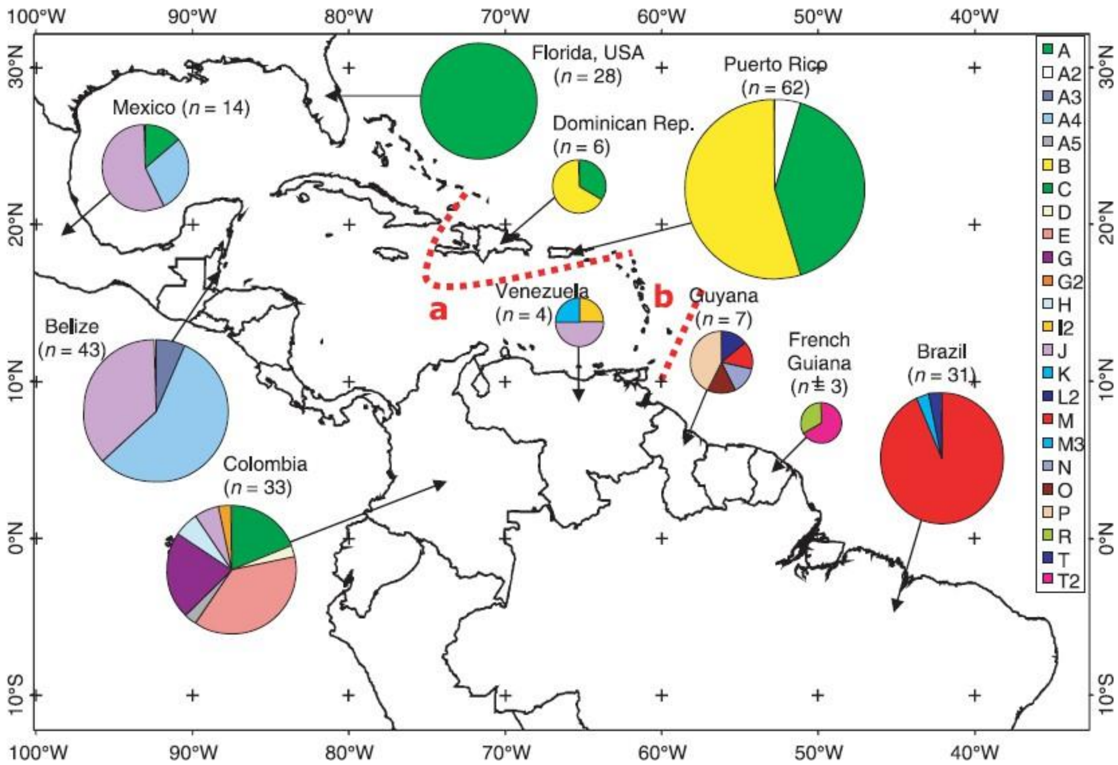

Figure 1: The three haplotype clusters found of *Trichechus manatus*. The circle area reflects the country sample size and disk (Vianna, et al., 2006)

2.2 DISTRIBUTION

The two subspecies of the West-Indian manatee (*Trichechus manatus*) have different distribution patterns.

The Florida manatee (*Trichechus manatus latirostris*) occurs along the coast and in several rivers of Florida all year around. During the non-winter months (March to November) some manatees migrate into waters along the Atlantic coast up to Virginia as well as along the coast of the Gulf of Mexico to Texas (Deutsch, 2000). In the winter period (December to February) the distribution of the Florida manatee shrinks to only warm waters of southern Florida.

The Antillean Manatee (*Trichechus manatus manatus*) is found in Atlantic tropical and subtropical coastal waters and rivers from the Bahamas to Brazil, comprising the Caribbean Sea as well as the Gulf of Mexico. In the Chetumal Bay (Mexico and Belize) a number of around 1700 manatees occurs there and constitute the main population of this species (Deutsch, et al. 2015).

Distribution Map

© The IUCN Red List of Threatened Species: Trichechus manatus – published in 2008.
http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T22103A9356917.en

Figure 2: Distribution of the West Indian Manatee (© IUCN)

2.3 MORPHOLOGY

Sizes and weights

Body weight:	Adult manatee: 300 kg - 800 kg
	New-born manatee: 18 kg – 25 kg
Total body length:	Adult manatee: 2,5 m – 4,5 m
	New-born manatee: about 1,20 m

Description

Like all other aquatic mammals manatees have evolved to fit into the constraints posed by the aquatic environment. Many aspects of their physiology, behaviour and anatomy represent adaptations for living in shallow water habitats. The general appearance of the Antillean Manatee is that of a massive streamlined body with a horizontally flattened and spatula-like round tailed fluke, very effective for propulsion. Like Cetaceans Manatees do not possess hind limbs, but well developed forelimbs or flippers. These flippers, with the elbow allowing remarkable flexion, are being used to "maneuver", to "walk" along the bottom, to scratch, to touch and even to embrace other manatees. Manatees also use the flippers to move food into the mouth and to manipulate food items (Caldwell & Caldwell, 1985). Each flipper has three to four nails.

There is no obvious neck visible and the head is relatively small with no external ear flaps. The small opening to the ear channel is located several centimetres behind each eye. Nostrils are located at the top and tip of the snout making it easier to breathe at the surface. They close automatically when a manatee submerges. The eyes are very small and located on the sides of the head.

Manatees have a prehensile snout that is used primarily to grasp vegetation but also allow them to manipulate food and objects. This short muscular snout is covered with modified vibrissae that have also a prehensile function in order to bring vegetation into the mouth.

The skin is extremely hard and thick (sometimes over 2,5 cm) and may provide some of the ballast needed to compensate positive buoyancy. Instead of having a blubber layer like other aquatic mammals, manatees have alternating layers of muscle and blubber. The whole body is covered by fine hairs (approx. 1 every 1 cm2) and it is suggested that they serve as sensory organs to detect water movements. Similar to elephants and hyraxes (manatees closest living relatives) manatees possess only molariform teeth especially adapted to chew vegetation with large amounts of abrasive material. This teeth (5-7) located in the back half of the lower and upper jaw, as they wear down, are being replaced by new teeth from behind. Tooth replacement is continuous, providing the manatees with a lifetime unlimited tooth production.

Figure 3: Manatee skull, lateral view (© Harald Wagner)

Figure 4: Manatee skull, dorsal view
(© Harald Wagner)

Figure 5: Lower mandibular of manatee
(© Harald Wagner)

The bones of the manatee are very dense and relatively heavy (pachyostotic). Pachyostosis increases specific gravity and seems to contribute to maintaining neutral buoyancy.
Adult manatees measure approx. 2,5 to 4,5 m in total length, with corresponding weights of 300 to 800 kg. New-borns weight 18 – 25 kg and measure about 1,2 m in length. However female manatees from Zoos are said to be heavier than males of the same length. The different position on the genital openings and the presence of axillary teats in the female allows visual differentiation of males from females. (Apart from the genital apertures there is no marked sexual dimorphism. In females there is a single teat in each axilla behind the flipper.)

2.4 ANATOMY

Digestive system
Because of the position of the diaphragm the gastrointestinal tract in manatees differ from the typical terrestrial mammal arrangement. The complete tract and the contents can account for up to 23% of the body weight.
Food is being gathered by using they very moveable and fleshy lips as well as the front flippers. Yamanski et al. (1980) determined that beside of mucous glands the tongue has a few taste buds and papillae which probably are used as tactile organs. The chewed food is passed into the muscular oesophagus, from there into the stomach via a marked sphincter with thick muscular walls. The manatee stomach looks like a simple sac with one noticeable digestive gland known as cardiac gland. This gland produces large amounts of mucus, hydrochloric acid and pepsin. The small intestine has a length of up to 20 m long in a large, adult manatee. The large intestine may be as long as the small intestine (can measure 20 m in length) and has a diameter, which is double as thick as the diameter of the small intestine. At the junction of the small and large intestine is a large, two-horned cecum. Manatees are hind-gut digesters, that means that more of the digestive process occur along the intestinal tract. The enlarged contains a rich microflora which enables them to digest cellulose and other carbohydrates. Food retention time in the digestive tract of Manatees is extremely long (146 to 170 hours). According to Reynolds (1980) manatees have similar digestive systems like other nonruminant herbivores, but with special adaptions.

Respiratory system
Manatee breath through two-valved nostrils located at the tip of the rostrum. A large obliquely sloped diaphragm separates the lungs from the abdominal viscera. The epiglottis is rudimentary. The manatee larynx does not contain true vocal cords but instead it is suggested that fleshy ligamentous fibro-elastic tissue may serve this function. The trachea splits into two bronchi which dissolve parallel and enter finally the lungs. The lungs are flat and elongated and extent horizontally along the back and may

be 1 m long. Harrison and King (1965) determined that the lungs have just several notches on their borders and are not lobed. The large air sacs consists of unusual large amounts of fibro elastic and smooth muscle tissues, which make sure to compress air into the lungs. Manatees replace about 70 % of the air in their lungs with each breath. Manatee normally tend to breathe every 2 to 5 minutes, but can stay under the water for up to 20 minutes. In order to conserve oxygen bradycardia slowly develops during diving. The ability of manatees to make long dives, in spite of relatively poor oxygen stores, is due to their low metabolic rate.

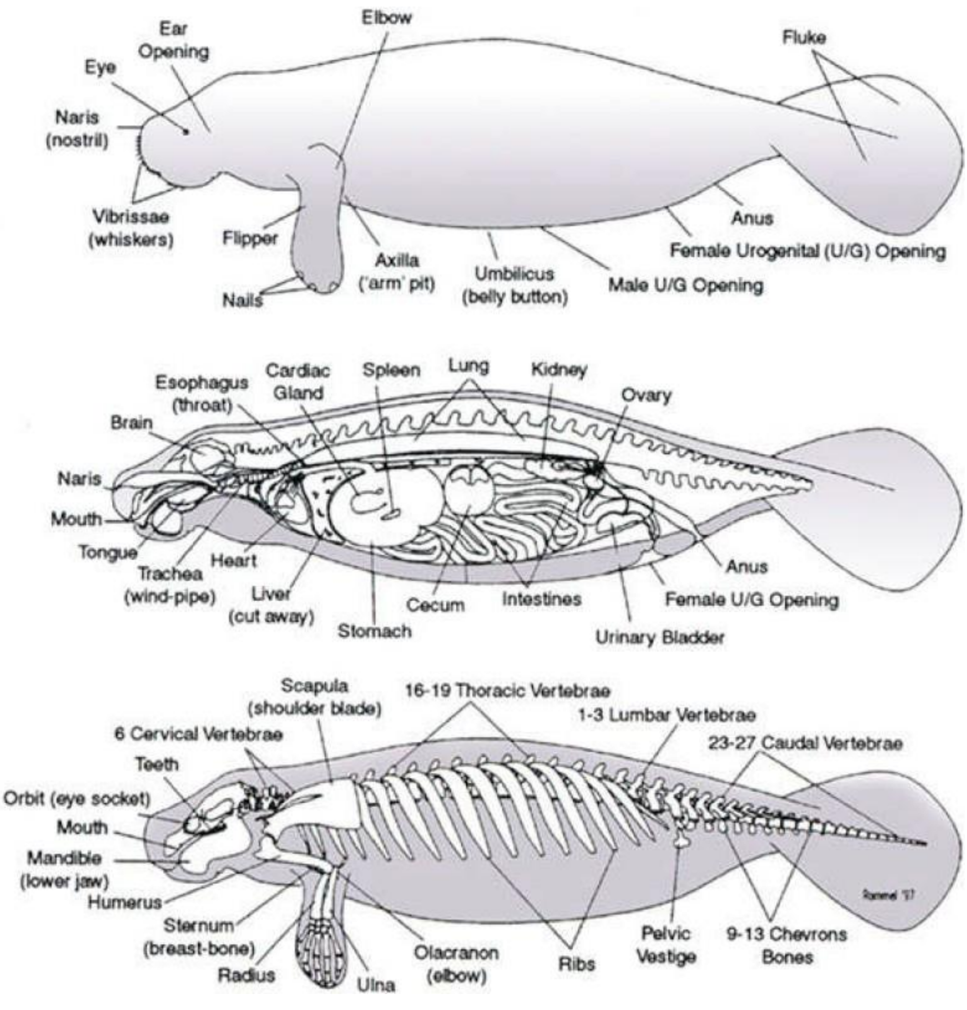

Figure 6: External and internal anatomy of the manatee (© Florida Fish & Wildlife Conservation Commission)

Circulatory system

Manatees have a globular, large heart with a deep interventricular cleft extending almost the full length of the ventricles. Furthermore the heart is different from other marine mammals in having a dorsally located left atrium. Normally the heart beats 50 to 60 times per minute (Harrison and King, 1965). A single opening in the atrium is the entrance for two venae cavae and two pulmonary veins. The posterior vena cava system is double. Throughout the whole body scatters unusual vascular bundles including the body walls, face and jaw, tail, and spinal canal (Husar, 1977b), as well as in the flippers, where they are most developed. These bundles are not comparable as networks of vessels like rete mirabile, but rather are "broomlike". Large arteries split into numerous small vessels, which run parallel for long distances and branch further.

Data about blood chemistry of manatees is provided by Walsh & Bossart (1999) in table 5, page 59.

In comparison to other diving mammals the haematocrit and oxygen binding capacity of the blood of Amazonian Manatee (*T. inunguis)* are low but similar to those for land mammals (Farmer et al. 1978)

Urogenital system
The kidneys of the manatee are only superficially lobulated and are located on the surface of the diaphragm. The ovaries are formed of large masses of beadlike spherules and lack a heavy capsular coat. The uterus of manatees is bicornate. The placenta is of the hemochorial, deciduate type. The testes are abdominal and their seminal vesicles are large. The nonglandular prostate consists of erectile muscular tissue (Caldwell and Caldwell, 1985).

Endocrine system
The hypophysis is oval shaped and has well developed anterior and posterior lobes. The Thyroid glands of tree individuals were investigated. The weight varies between 0.11 to 0.13 g/kg of body weight. Furthermore low thyroid activity is suggested by the presence of profuse colloid. Uncommon tyroid structure is suggestively linked to the pachyostotic bone, the low rate of oxygen consumtion and the sluggish behaviour of manatees. The medulla of the thymus is small and pale and the cortex is prominent. The retroperitoncally located adrenal glands are between the carotid artery and bronchus and show an atypical histology. The spleen is small. (Husar, 1977b)

2.5 NERVOUS SYSTEM AND SENSORY SYSTEMS

Adult manatee have a relatively small brain that weighs approximately 370 g. This weight is considerably less than the brain weights of a dolphin or sea lion of equivalent body size. Both hemispheres are separated by a deep longitudinal fissure and lack extensive convolutions. The structures found in the manatee brain are the same as those found in most mammals. However the relative importance of different regions is distinctive. For example it is noticeable the large trigeminal (cranial nerve V) and facial (cranial nerve VII) nerves associated with the facial vibrissae.

2.5.1 Audition, Sound Production and Communication

Manatees auditory system has been studied in several occasions since Hartmann´s (1979) first claim about their exceptional acoustic sensitivity. In turbid environments, with limited visual cues, sound is the most efficient way of communicating. Hearing capabilities have been investigated in different ways. Some studies (Ketten, et. al. 1992) have shown that anatomical specializations of the ears and the skull support the idea that auditory input is an important source of information about the environment but also enables the manatee in determining the direction from which a sound comes. Ketten also suggested adaptations for low frequency hearing. Reliable behavioural audiograms of two captive Florida Manatees defined the frequency range of best hearing to be from 6 kHz to 20 kHz, with a peak sensitivity between 16-18 kHz (Gerstein, et al., 1999). However manatees are capable of hearing sensitivities up to 90,5 kHz (Gaspard, et al., 2012). However it is important to remark that frequencies outside the 6 – 20 kHz requires noticeable increases in sound pressure levels. Other studies using auditory evoked potential techniques suggested that manatees had greatest hearing sensitivity at lower frequencies (Bullock, et al., 1982).

Hartmann (1979) qualitatively described Manatee calls as squeaks, chirps and grunts and described the behavioural context in which these calls were produced. Recent studies describing the vocalizations of manatees suggest the presence of two different vocalizations types: tonal harmonic calls and broad-band, less tonal calls (Nowacek et al., 2003; Sousa-Lima et al., 2008). Even if most of the studies done so far are based on the Florida Manatee there are no obvious reasons to believe that Antillean Manatees behave differently.

It has been shown that adults of both sexes produce vocalizations at rates that vary with activity and behavioural context. The lowest rate has been registered during resting, intermediate during travelling and highest at nursing and other mother-calf interactions. In general communication calls are quite common between cows and nursing calves.

2.5.2 Tactile Senses

Manatees possess an array of hairs and bristles on the face distributed in a well-defined pattern involving nine distinct regions (see Figure 8) (Reep, et al., 2001). These hairs are being used by manatees in tactile exploration and in grasping behaviour (Bachteller & Dehnhardt, 1999). In conjunction with a well-developed and modified facial musculature this bristles function as a prehensile system to obtain, manipulate and ingest vegetation. Studies done with captive manatees demonstrated that this prehensile organ conveys the manatee the ability to resolve fine textural differences and to detect particle displacements of less than a micron (Bachteler & Dehnhardt, 1999; Bauer, et al., 2010).

Reep, et. al. (2001) found out that the hairs and bristles of the manatees have a similar sensitive microanatomy as the vibrissae of other mammals, characterized by a prominent blood sinus complex and substantial innervation. The data suggest that manatees perioral bristles might have a similar sensory role as vibrissae in other mammals. In addition it was shown that the whole complex of facial hairs and bristles play an important role in hydrodynamic reception (Gaspard, et al., 2013). Using a Go-No Go training procedure the authors could demonstrate that manatees can detect particle displacements of less than 1 micrometer for frequencies of 50-150 Hz and less than a nanometer at 150 Hz. It was also shown that the facial vibrissae were responsible for this detection of hydrodynamic vibrations.

Figure 9: Vibrissae (© v. Fersen)

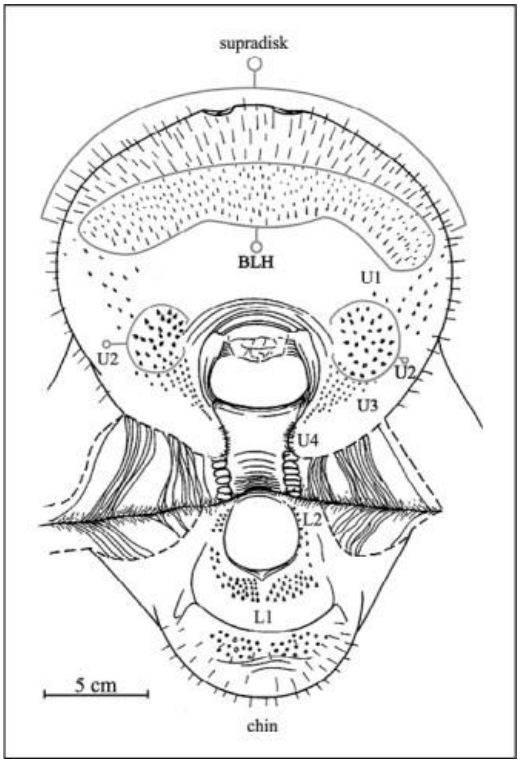

Figure 8: perioral region, showing the location of the upper (U1-4) and lower (L1-2) bristle fields, bristle-like hairs (BLH), supradisk, and chin. Dotted lines indicate cutsmade in the cheek muscles to allow a fuller view of the oral cavity. (© Reep *et al.*)

Manatees also have sparse hairs scattered over its body decreasing in density from dorsum to ventrum. These hairs are anatomically similar to the sensitive facial hairs and also have unique features of innervation. It is assumed that these hairs constitute a distributed tactile system capable of perceiving and detecting water movements and are used by manatees to navigate, showing interesting similarities to the lateral line of fishes. It is believed that manatees developed this interesting sensory system in compensation for the reduced availability of visual cues.

2.5.3 Vision

Manatees use vision even if this sensory modality might be limited by environmental constraints like turbid water. Vision is a product of multiple, integrated stages of processing from optics at the level of cornea and lens, to physiology at the retina level and finally to the level of perceptual processing and cognitive analysis. Regarding optics the eye of the manatee is rather small (13-19 mm diameter) and is set within the ocular fascia (Mass, et al., 1997). The eyeball is almost spherical, the lens is of lenticular shape. In contrast to the eye of the majority of mammals featuring avascular corneas, the manatee cornea has small blood vessels. Underwater the eye is almost emmetropic or slightly hyperopic. In the air the eye is markedly myopic (Mass & Supin, 2007). It is unknown if manatees has some mechanisms to compensate aerial myopia.

A detailed description of the retina showed that the laminar structure of the retina is well developed (Cohen, et al., 1982). Using light and electron microscopy, they found that both rodlike and conelike photoreceptors are present. The two cone sublcasses found suggest the possibility of colour vision.

Figure 10: Small eyes and right flipper with nails of a manatee (© v. Fersen)

Regarding visual acuity Mass et al., (1997) indicated that manatees had limited visual resolution, 20 min of visual arc. However Gerstein (1994) reported that manatees detect and discriminate shapes, sizes and some pattern differences. Another experiment done with two captive manatees showed not only individual differences regarding their visual acuity but also confirmed previous data about the limited resolution in manatees (Bauer, et. al., 2003). The authors assume that manatee vision is probably not of great utility in evaluating fine details of objects, especially at close distances. It has been suggested that manatee use vision at intermediate or longer distances to orient toward larger objects. Colour discrimination experiments showed dichromatic colour vision. Griebel and Schmidt (1996) showed that manatees could discriminate blues and greens from grey, but not reds or blue-green. This result is in agreement with morphological data presented earlier about the presence of rods and cones in the retina. The presence of both rods and cones suggest that manatees use vision at night and in daylight.

2.5.4 CHEMORECEPTION

Similar to other marine mammals, the olfactory areas of the brain and the olfactory bulb in manatees are reduced. Manatees have taste buds on their tongues and it has been suggested that they may function as receptors for environmental chemical cues, like freshwater or pheromones. Hartmann (1979) and other authors have hypothesised that manatees during the mating season use pheromones to identify females ready to mate. Chemoreception may also explain food preferences in manatees kept in Zoos.

2.6 PHYSIOLOGY

Respiration rate varies according to the activity, water temperature and reproductive status. Normally they rise to breath every 1 – 8 minutes but can stay under the water for up to 24 minutes (Reynolds, 1981).

In contrast to marine mammal species that show a high metabolic rate and a thick blubber layer in order to preserve heat allowing them to live in cold-water habitats, manatees have a remarkable low metabolic rate. In fact manatees exhibit metabolic rates as low as 25% of that predicted on the basis of body mass (Gallivan & Best, 1986) making them unique among marine mammals. One main reason for that can be found in the diet of manatees. The aquatic plants and algae they consume consist of 80-90% water, resulting in low energy density and therefore providing low caloric intake (Best, 1981). It is suggested that the low metabolic rate is an adaptation of a large tropical animal that only consume relatively poor quality of food. Limited heat production and high conductance lead to poor heat storage (Irvine, 1983). This physiological constrain determine the distribution of this species. In consequence manatees, especially those living in Florida where cold-water columns are frequent in winter months, require the access to warm water sources. Prolonged exposure to water temperatures below 20°C can cause a condition called Cold Stress Syndrome (CSS), that in most cases result in death. In a study where the pathological features of CSS were characterized Bossart et al. (2002) found symptoms that include emaciation, fat store depletion, skin lesions, dehydration, constipation and other secondary infections. CSS at the behavioural level can include shivering, changes in resting patterns and cessation of feeding (Walsh & Bossart, 1999).

When looking at the distribution pattern of the Manatee, that normally move from marine to fresh water habitats and vice versa, it is noticeable that this adaptation can only be managed through physiological capabilities related to water balance and osmoregulation. Even if the Manatee is most frequently associated with fresh water, individual animals spend prolonged periods at sea, as suggested by the amount of barnacle growth present on their bodies (Husar, 1977). The manatee´s ability to concentrate its urine (and thus conserve water) suggests that they may consume sea water to maintain fluid homeostasis (Irvine, et. al., 1980). Though, Ortiz et al. (1999) using water turnover studies showed that manatees exposed only to sea-water and sea grass do not drink sea water voluntarily. They eventually refuse to eat sea grass. It seems that their water needs are met from metabolic water production, utilizing body fat as a "water storage depot", as do other mammals. Water flux studies have shown that manatee consume high quantities of water (Ortiz, et al., 1999). The authors suggest that a 500 kg manatee living in fresh water system would drink 20,5 l. of water per day. They also demonstrated that osmolality and electrolyte concentrations in wild fresh-water manatees are similar to those of wild and zoo animals living in salt water. This data suggest that wild animals in fresh-water system search for salts by consuming plants with sufficient salt content. It also means that manatees living permanently in fresh- water systems, like in Zoos, may be susceptible to hyponatremia (Ortiz, et al., 1999).

Compared to other marine mammals detailed diving behaviour and physiology has not been described in detail in manatees. One study by Gallivan et al. (1986) showed that Amazonian Manatees in an unrestrained situation showed a constant heart rate during diving and only exhibited a small tachycardia during breathing. Forced dives caused a marked bradycardia. The investigation also demonstrated that manatees are capable to dive beyond 10 minutes duration without the need to resort to anaerobic metabolism. Even after long dives they recover within 3 – 4 short dives. It is suggested that their low metabolic rate is responsible for these long dives.

2.7 REPRODUCTION

Reproductive organs

While the reproductive anatomy of manatee females has been described extensively (Marmontel, 1988), there are only few studies regarding males. In females the ovaries are situated just postero-lateral to the kidneys. They are oval in shape and are remarkable because the active cortex is a two-dimensional layer on one side of the ovary (Bonde et al., 1983). Manatees have a bicornuate uterus with the ovaries ovulating with equal frequency to either uterine horn (Marmontel, 1988). Males' penis is about 30 cm in length. The mean testicle diameter is 7,5 cm and the testis appears to be divided into two or three lobes. Like most other marine mammals the testis are located internally.

Sexual maturity

Female manatees reach sexual maturity between 2.5 and 6 years of age. Male manatees reach sexual maturity between 2 and 11 years of age. It is important to note that these data refers to the Florida manatee.

Gestation period

The gestation period is about 12-14 months (White et al., 1984, Odell, et al., 1995; Reid et al., 1995).

Birth

Normally, the calf is born tail first and measures about 1,2 m with a weight of 18 – 25 kg (White, 1984). Manatees calve approximately every two and half year to three years.

Calf nursing behaviour

The calves nurse from one of the two nipples located in each axilla behind the flippers. Normally the calf and the mother float horizontally and the calf swims in the direction of the nipple. The only assistance of the mother is given by the fact that she swims slowly holding her flipper out of the way. Normally weaning occurs after the calf is 2 years old, but start eating solid food (for example lettuce) when 40 days old (Moore, 1957). In Zoos cows have been observed to nurse their own calf and other much larger calves that are not her own. It has also been observed that calves are very curious and exploratory and start ingesting vegetables and algae at very young age (approximately 1-2 weeks); these pieces are actually passed intact in the faeces in the first few days but then start immediately being digested.

When natural suckling does not immediately occur, 2 captive females have been observed to maintain milk in the glands that are often "massaged" with the flipper of the female and lactation has then naturally occurred at 39 and 40 days after birth without any mammary gland complication.

All these information can be currently collected and made available in zoos and aquaria with underwater windows through constant observations of the dyads for prolonged periods after birth.

Figure 11: Calf nursing behaviour (courtesy ZooParc de Beauval)

Sex differences
Sexes can be distinguished externally by the relative position of the genital aperture to the anus. In females the genital aperture is closer to the anus, in males the genital aperture is in closer proximity to the umbiculus in the center of the abdomen.

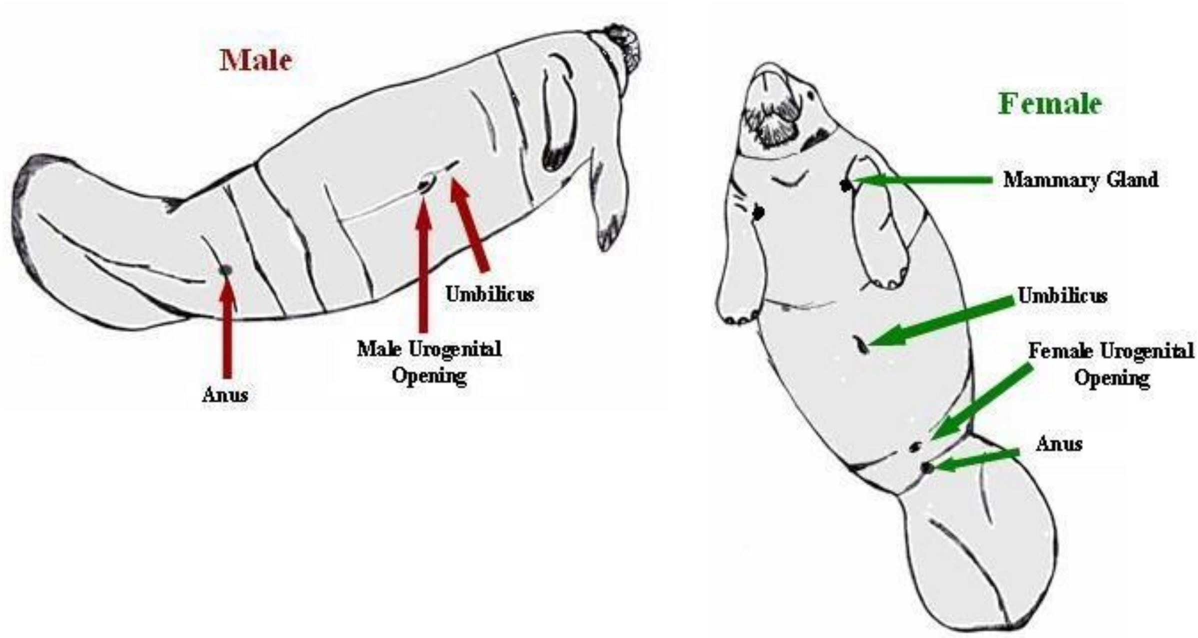

Figure 12: Sex differences in manatees. (© Florida Fish & Wildlife Conservation Commission)

2.8 BEHAVIOUR

The behaviour of manatees is simple and well suited to the needs of these animals. Manatees evolved in regions with plentiful food supplies, fairly constant temperatures, and no natural predators. Because of this they have never needed to develop complex social behaviours to obtain food or protect themselves. (Hartman 1979).

It is perhaps this description of the behaviour of manatees that summarizes part of manatees´ life.

Activity
Manatees can swim great distances. According to Deutsch et al., (2003), that tracked remotely manatees during their seasonal movements, registered distances between the northerly warm-season range to the southerly winter-range of 280 to 830 km. Normal swimming speed is about 2-7 km per hour. Manatees are neutral buoyant and rest at different depths in the water column. Observations suggest that they can move vertically in the water by changing the volume of air in the lungs or intestines (Rommel & Reynolds, 2000).

In general terms the activity pattern of manatees, like those showed by big herbivorous animals is mainly affected by environmental constraints. Whereas the activity budget is regulated by changes in the relative energy intake and expenditure. Manatees feed for 6 to 8 hours per day (Hartmann, 1971; Reynolds & Odell, 1991). During that time manatees consume about four to nine percent of their body weight (Etheridge, et. al., 1985). Daily activities consist of feeding, resting, travelling and socializing. The relative importance of the various behaviours within a day expressed as total time spend engaging in these behaviours depends on the efficiency and availability of energy and nutrient intake. During the day manatees select certain habitats for feeding or resting. Between feeding bouts, manatee normally spend 2-4 hours for resting.

Most data suggest that manatees do not have a well-defined circadian rhythm. According to several studies (Hartmann, 1979; Montgomery, et al., 1981; Reynolds, 1981), manatees do not show differences in their activities during the day. However, other studies showed greater activities during the day compared to the night (Henaut, et al., 2010; Holguin-Medina, et al., 2015).

Spatial use and activity pattern of the Homosassa Springs Wildlife State Park (HSWSP) captive manatees apparently were influenced by energy constraints and nutrient intake by provisioned food availability over the day and natural vegetation over the study period (Horikoshi-Beckett & Schulte, 2006)

Social behaviour

Manatees are considered a semi-social species, meaning that they do interact with other individuals, but not on a long-term basis (Hartmann, 1979). They primarily show strong bonds only between mothers and their calf. This bond persists for aprox. 1 to 2 years, even if nursing behaviour might continue for up to 3 or 4 years (Wells et al., 1999). During this period the calf learns relevant environmental information and social skills from the mother. Unlike terrestrial herbivores the herd structure and social organization of manatees do not seem to be as strictly defined (Hartmann, 1979). Harper & Schulte (2005) refers to a semi-social situation in which the numbers of individuals vary considerably and members shift from one group to another. Groups are normally composed by 4 to 25 animals (Rathbun, et al., 1995). Reep and Bonde (2006) found out that these transitory groups consist of adult males pursuing one or more receptive females and may remain together for periods of up to a month. Calving interval vary individually but it is assumed to be between 2 to 3 years.

Sexual behaviour

It appears that manatees breed year around, at least from what is known from South Florida (Reynolds, 1977). However Reid et al. (1995) observed some peaks in calving and reproductive activity in spring months. Larkin (2005) suggest that manatees shows reproductive flexibility depending where they live. It is still unknown if manatees are seasonally polyoestrous or have a long oestrus period (Reynolds, 1977).

Manatees are promiscuous, with multiple males mating with individual females. This suggests that manatees are sperm competitors. Surprisingly, manatee testes are not as large as expected for a sperm competitor. For adult males in non-winter, testicular size is approximately twice what is expected, based on allometry, for "typical" (*i. e.*, non- sperm competitor) male mammals of similar size (Reynolds, J. et al., 2004). This aspect should be taken into account when keeping these animals in Zoos. Thus, keeping many males in a facility with only one or few females might promote breeding.

Observations indicate that once a female becomes sexually mature and comes into oestrus, male manatees form a mating herd pursuing the female. The female is normally escorted by the males for a period of 2-4 weeks (Hartmann, 1979). The 2-4 weeks of male pursuit period may be a result of the establishment of dominance relations among males and is related to the sperm competitors' strategy. During mating, both male and female lie horizontally in the water in a ventral to ventral position (Hartmann, 1971). Precopulatory activity in manatees is intensive and prolonged and presumably serves some physiological purposes. Similar to elephants, female manatees may have several sterile oestrus periods prior to successful conception (Hartmann, 1979).

In Appendix 1 an ethogram showing different behavioural categories of an Antillean Manatee is presented (Gomes, et al., 2008).

2.9 Diet and Feeding behaviour

Manatees are aquatic herbivores, something rare among marine mammals. They spend 4 to 8 hours per day feeding and normally consume an amount of plants corresponding to between 5 to 10% of their body weight. Their digestive system is similar in structure to that of the horse. They are hindgut fermentors (it means that the site of fermentation is at the end of the digestive tract) and therefore have very large intestines (approx. 20 meters long) representing 14 percent of the body weight. Depending on the area where they live Manatees utilize over 60 different species of marine and freshwater plants. These plants are of a relatively low quality: high in fiber, but low in protein and caloric value. Manatees maximize the efficiency with which they extract nutrients by having a very slow gut transit time of 146 hours (six days).

Although the plants eaten by manatees contain approx. 60-95% water and therefore mean an important fresh water source, these animals need regular access to freshwater. Manatees also eat invertebrates, perhaps unintentionally (Best, 1981). In Zoos they have been observed to accept fish.

It is still unknown if manatees need to drink freshwater. However there are many observations where they have been spotted drinking freshwater. Functional morphology indicates that the kidney should be able to produce hyperosmotic urine, allowing the animal to eliminate the excess of salt during seawater ingestion.

2.10 LONGEVITY AND POPULATION PARAMETERS

Despite the fact that manatees have been intensively studied during the last 30 years no information is available on the lifespan. The oldest animal recorded is Snooty, born in 1948 and still alive in the South Florida Museum´s Parker Manatee Aquarium. Although it is known that manatees can live as long as 60 years the average age at death of adults recovered in Florida (USA) is 12 years and only 1 percent of animals recovered are older than 30 years. Given the fact that 86 percent of the manatees reach sexual maturity by age three to five and 73% of dead animals were seven years or younger it is evident that many animals do not even come close to realizing their full reproductive potential. It also means that only a relatively small number of females are producing most of the calves. Marmontel et al. (1996) showed that the earbones of manatees have growth layers that seems to persist throughout the animal´s life and therefore can be used as an indicatos of age. Using this technique Marmontel demonstrated ages of over 50 years in manatees.

2.11 FIELD DATA, CONSERVATION STATUS, THREATS

Habitat

Trichechus manatus manatus is found in diverse habitat like rivers, lakes, coastal and inland lagoons as well as coastal marine environments, including seagrass, mangrove, and coral reef ecosystems. They can live in fresh, estuarine water and marine water for extended periods of time (Deutsch *et al*, 2015).

Population

According to the IUCN 2600 individuals of *Trichechus manatus manatus* exist currently but scattered widely through the Caribbean region and the northern Atlantic coast of South America. The estimated total population size of 4100 manatees is the average of the counted population number of 2600 and the optimistic estimated suggestion of 5600. The age structure of this population is unknown. The IUCN assume that the percentage of mature individuals lie somewhere between 46% and 70%. Because of this estimation the IUCN assume that the overall number of mature individuals in this subspecies is likely to be less the 2500 (Deutsch *et al*, 2015).

Table 1: Population numbers in different countries

Table 1. Summary of reported data by country for extant manatee populations.

Trend: I=Possibly Increasing; S=Likely Stable; D=Probably Declining; U=Unknown due to data deficiency.

MinPop: Minimum Population Counts/Estimates based on best available data.

*Only two countries, Mexico and Belize, reported population estimates of ~1,000 or more animals. Prime manatee habitat (Chetumal Bay) is contiguous along the border between these countries, increasing the uncertainty related to these population estimates.

Country	Trend	MinPopEst
Bahamas	I	5
Belize	S/D	700
Brazil	S/D	200
Colombia	U/D	100
Costa Rica	D	30
Cuba	U/D	50
Dominican Republic	D	30
French Guiana	S	10
Guatemala	U	50
Guyana	D	25
Haiti	U	5
Honduras	S	50
Jamaica	U/D	25
Mexico	U	1,000
Nicaragua	D	71
Panama	U	10
Puerto Rico	S	128
Suriname	D	10
Trinidad & Tobago	D	25
Venezuela	D	25
Total (n=20)		**~2,549**

Conservation status

According to the IUCN Red List of Threatened Species 2016-1, the Antillean manatee *(T. manatus manatus)* is classified as Endangerd. According to CITES *Trichechus manatus* is listed in Appendix 1.

Threats

Major threats for *Trichechus manatus manatus* are habitat degradation and loss, hunting, incidental catch/accidental take, watercraft collisions, entanglement in fishing gear, pollution, natural disasters, and human disturbance. Hunting is diminishing in some areas, all other threats are increasing in most areas. Illegal hunting for subsistence and profit was reported as a significant threat in Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, French Guiana, Guatemala, Honduras, Mexico, Suriname, Trinidad and Tobago, and Venezuela. Pollution from agriculture and mining was consistently noted in reports from South American countries. Intrinsic factors that limit the population's ability to withstand these anthropogenic impacts include low fecundity, slow growth, limited dispersal, and restricted range (Deutsch *et al*, 2015).

SECTION B: MANAGEMENT IN ZOOS AND AQUARIUMS

MANAGEMENT IN ZOO AND AQUARIUMS

2.1 ENCLOSURE

Manatees, like Cetaceans live their entire life in water and they do not need a dry resting surface. Enclosure sizes are set to satisfy the need of manatees allowing the animal to show natural behaviours at all ages. It should be large enough for the animals to achieve the full range of body motion and physical movements normally performed, and it should contain furniture to physically and psychologically enrich the environment and stimulate normal physical movement and behaviour of the animals. In case of outdoor enclosures it is recommended to provide some sort of shelter from natural weather conditions such as for example sun.
Obvious changes in zoo exhibit design have been occurred in the last decades, not only to please human perception in terms of space and surroundings but, most important, to address and incorporate the increased knowledge of the species specific needs.

2.1.1 POOL - SHAPE, INDOOR/OUTDOOR, DIMENSIONS

A wide variety of irregular pool shapes are recommended. If possible avoid geometric contour shaped pools (like circle, square, oval). They have the advantage over irregular pools as they provide better water flow, but are not as suitable in order to create a naturalistic environment. Furthermore circular shaped pools seems to leave the impression in the public that the animal show stereotypical swimming. It is recommended to construct enclosures with different depths of water. Manatees not only like deep water but also shallow areas.

A wide variety of building materials can be used for the construction of the manatee pool. The principal requirements are that the pool should be water tight, non-abrasive, easily sanitized and resistant to puncture. Concrete pools for housing manatees proved to be the best material.

Two separate pools are recommended and mostly needed. One medical pool and one general holding pool. The medical pool has to be designed and constructed in a way that allows rapid water drainage and easy access to the pool from outside the building (for example in the case of animal transports). Moreover when building a manatee enclosure it is important to consider having different sections or divisions in the main holding pool allowing the division of the pool trough nets or other barriers. In this way animals can be separated when needed.

It also needs enough space for the staff to move and work around the animals avoiding traumatic risks arising from the size and movement capability of the animals.

Indoor pool

Air and water temperature in indoor facilities should be regulated by heating or cooling systems in order to protect manatees from extremes. Rapid changes in air and water temperatures should be avoided. Water temperature should be kept between 25°C and 30°C. Prolonged exposure to water temperatures below 20 °C can lead to the Cold Stress Syndrome and death. Air temperature in indoor pools is best at 26°C – 30°C.

The indoor enclosures should be ventilated by natural or artificial means to provide a flow of fresh air for the manatees and to minimize the accumulation of gases and noxious odours. A vertical air space averaging at least 2 meters should be maintained in all pools housing manatees. The indoor housing facilities for manatees must be provided by a regular diurnal lighting cycle of preferably by natural light. The size requirements of the pool can be found below.

Outdoor pool

In case of outdoor pools, caution has to be taken in order to keep air and water temperature comparable to those defined for indoor pools (see paragraph indoor pools above). A manatee should not be moved to an outdoor enclosure when it is not acclimated to the air and water temperature ranges for the outdoor facility. Furthermore, when housing manatees in an outdoor enclosure it is important to keep the water temperature between 25°C and below 30°C. The size requirements of the pool can be found below in 1.1.2 Dimensions.

Dimensions

Defining the size of enclosures that secures the welfare of the animal that has to live in it is not an easy task and can be approached from two different standpoints. First defining the minimum size and volume of an enclosure for a certain number of animals that proved to be non detrimental to the group. Second recommending size and volumes based on best practice. In this Husbandry Guidelines we will present data reflecting official minimum standards from countries like USA and Germany.

According to the United States Department of Agriculture (USDA, 2001c¬). The minimum space requirement according to the USDA is based on the minimum horizontal dimension (MHD), depth, volume and the surface area of the primary enclosure (USDA, 2001c; USDA, 2001f). The MHD is the diameter of a circular pool, the required MHD is two times the average adult length of the longest specimen housed in the pool (USDA, 2001g).

The minimum depth of the pool must be 1,52 meters (5 feet) or ½ the average adult length of the longest specimen housed therein, whichever is greater. According to the USDA the average adult length of a manatee is 3,51 meters. Those parts of the enclosure which do not meet the minimum depth requirements cannot be included when calculation space requirements for sirenians. (USDA, 2001c)

If the pool meets the MHD and the depth requirements, then the pool will have sufficient volume to house up to two sirenians (USDA ,2001g). If additional sirenians are to be added to the pool, the volume as well the surface area has to be adjusted to allow for the additional space (USDA, 2001c). The volume of water required for each additional manatee is 35, 50 m3. (USDA, 2001c). In the same document the required surface areas are given for marine mammals, the required surface area for one manatee is 14,47 m2. This means that for each manatee added the surface area should be increased with 14,47 m2. (USDA, 2001c)

Even if the American standards might find acceptance within the USA and are addressed for another subspecies T.m. latirostris, for Europe we rather recommend to follow the new German Guidelines that meanwhile are also used as reference for many new legislation in Europe. According to the new German Standards and Guidelines (Gutachten über Mindestanforderungen an die Haltung von Säugetieren, 2014) the manatee enclosure should consist of at least two separate pools. One of these pools can be used as a medical pool. Regarding dimensions: the new Minimum Standards establish for a group of two animals a minimum water surface of 150m2 and a volume of 270m3. The depth should varies from 0,7 m to at least 3 m. For each additional animal an increase of 25m2 in water surface is required with an equivalent additional volume varying between 50 and 75 cubic meter.

A medical pool should be equipped with a false bottom (highly recommended) or with a system that allows draining the pool when needed. Regarding size the medical pool should be at least 4 metres long, by 2 metres wide and 1metre deep. It is important to remark that the medical pool is only for a temporary accommodation (i.e. for medical treatment)

It is important to mention that measurements are a significant part of the general exhibit requirements for every animal species. They are as important as environmental and behavioral enrichment. These standards only lay down general principles of animal keeping, to which the members of the Marine Mammal TAG feel themselves committed.

Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size of enclosures. The Marine Mammal

TAG strongly recommends that users of this information consult with the editors and individual species EAZA program coordinators in all matters related to analysis and interpretation of the data, especially before implementation

2.1.2 Enclosure barriers and partitions

In general, enclosure barriers and partitions within the enclosure have to be maintained in a condition which presents no likelihood of harm to the manatees. Enclosure barriers should be designed and constructed to keep the public at considerable distance from the animals.

Even if manatees are completely aquatic and usually do not leave the water, caution has to been taken in cases where manatees “walk” on land. Sometimes manatees “walk” on their flippers and can surpass gates moving from one pool partition to the other. Therefore in the case of partitions or even secondary or auxiliary pools it is recommended to keep the upstand 20 cm above the water surface.

For the division of space within the pool several materials can be used: concrete walls with gates, strong nets that can be attached to fixed points in the pool wall, glass, acrylic or wooden fences. It is important to remark that manatees are strong animals and in cases where manatees experience stress (like separation or capture) they will be able to knock down barriers, that are not well anchored, or even pull down nets.

2.1.3 Pool walls and bottom

The walls and the floor should be free of gaps and spaces, which could house micro-organisms. All surfaces must be free of sharp projections i.e., screws, nails or wire, which are a potential cause of injury. Rocks and trees installed in the enclosure should allow normal movements of the animal and must be well anchored.

It is important that the bottom of the pool has a pitch towards the drains.

2.2 Furnishing and maintenance

Water and Power Supply

As manatees require constant water and air temperatures, reliable sources of water and electric power is required. There must be an emergency power generator and secure water supply available all the time.

Storage

Food items must be stored in rooms that adequately protect such items from deterioration, spoilage (harmful microbial growth), and vermin or other contamination. Refrigerators must be available for perishable food, such as fruits and vegetables. No substances that are known to or may be toxic or harmful to manatees should be stored or maintained in the food storage or preparation areas. Exceptions are cleaning agents; these may be kept in secured cabinets designed and located to prevent food contamination.

Waste disposal

Provision must be made for the removal and disposal of animal and food wastes, trash, and debris. Disposal facilities must be provided and operated in a manner that will minimize odours and the risk of vermin infestation and disease hazards.

2.3 ENVIRONMENT

2.3.1 TEMPERATURE

It is recommended to keep manatees in pools with a minimum water temperature of 25°C and a maximum of 30°C. Because manatees cannot deal with cold water temperatures for long periods it is important that the minimum temperature of the pool is above 20°C (Cold Stress Syndrome). Although, air temperature is not as crucial as water temperature, caution has to be taken especially in the case of outdoor enclosures. It is recommended in this case to keep the temperature range between 25°C and 30°C. In indoor enclosures the air temperature is normally regulated and is kept in values that are comparable with those registered in the water.

2.3.2 VENTILATION

Indoor enclosures should be designed to provide a relatively uniform distribution of fresh air, and to avoid unnecessary draughts over the enclosure. The incoming air should be fresh, not recirculated. The volume must be such as to assure an adequate supply of oxygen, reduce noxious or unpleasant odours and dilute airborne pathogens.

2.3.3 LIGHTING

Indoor housing facilities for manatees should have lighting. This can be done preferably with natural means. If artificial lighting is used, caution should be taken to mimic the natural spectrum and photoperiod.

2.3.4 NOISE

Given the fact that Manatees possess an "exceptional hearing sensitivity" caution has to be taken when enclosures are being constructed near "noise polluted" areas. Even if most of the ability to detect sound was tested in water, sirenians are also known to be capable of detecting some airborn sounds. Circulating water systems produce background levels of noise from pumps and other technical equipment. Normally manatees seem to adjust to these droning sounds without difficulty. However some individuals respond adversely to loud piercing sounds, which are all too frequent during renovations. Noise from even distant construction activities might be transmitted with little attenuation through the ground and into the pool. Under certain conditions, the behaviour of some animals may be noticeably affected and they may go off feed.

2.4 Water treatment

Because manatees spend their entire life in the water, their quality is crucial to ensuring their health and well-being. Good water quality is not always visible and does not necessarily mean clear water. Critical water characteristics include concentrations of dissolved oxygen, stable and appropriate pH level, absence of toxic chemicals, low bacterial counts, salinity, temperature, Etc.

It is important to point out that all the details and steps involved in the treatment of water described hereafter refer to a closed system, in which the water recirculate.

In a controlled environment an optimal water environment can be assured through the quality and functionality of a properly designed and installed Life Support System. The process of achieving optimal water quality has gone through a continual evolution in definition and techniques. This is partly due to the fact that our understanding of the water quality needs of aquatic mammals in general has evolved. Finally and especially because of the diversity of operating systems and equipments that are being used in Zoos it is nearly impossible to define the optimal water treatment system.

Filtration and Sterilization are the two processes that are responsible for good water quality. In general filtration eliminates undesirable material from the circulating water and concentrates this material in the filter media. Sterilization refers to the nonselective reduction of all microorganisms in order to control potential and known pathogenic organisms.

Besides filtration and disinfection, another important aspect that is crucial for a good water quality is the turnover rate. It refers to the amount of time it takes for a system to pass the whole volume of water through its system once. Based on the experience of many zoos keeping manatees a turn over rate of maximum two hours is recommended.

2.4.1 Filtration and drainage

Optimum water circulation is essential when filtering water holding large aquatic mammals like manatees. An important prerequisite is to suspend solids so that they reach the pool drains, skimmers and finally the filter. Caution has to be taken to avoid the accumulation of solid sediments on the bottom of the pool and a good flow through the whole exhibit avoiding “dead zones”.

In the case of manatees it is highly recommended that the water flow passes a pre filtration process before entering in the mechanical and also the biological filtration process. Pre-filters are designed to remove vegetative matter and other bigger particles suspended in the manatee pool. Due to the fact that manatees feed on several types of vegetative matter in high quantity (approx. 25 kg/day/animal), the amount of solid material left over in the water is quite high, therefore a good working pre filtration process is necessary to relieve the next filtration step.

When designing a Life Support System for manatees it is best not to copy other systems developed for other marine mammal species (like dolphins, sea lions), because the bio load produced by vegetation food use and abundant faecal production is substantial and very different from all the other species. One must use rather specialized LSS designed for the treatment of waste water or for animals like hippos, elephants etc., and moreover absolutely seeking expert advise beforehand, due to the cost involved in construction and subsequent maintenance. All these systems have to deal with water with large and high quantities of organic material.

Adequate drainage must be provided for all enclosure pools and must be located so that all the water and organic material contained in such pools can be effectively eliminated. Usually most of the water is being drained using an overflow drainage carrying the suspended solids from the upper water column (approx. up to 80% of the whole volume). The remaining 20% of the water volume is being drained by using one or more bottom drains, located if possible in the deepest part of the pool. In cases where exhibit design constraints result in the formation of “death zones” or interrupt an optimal water flow a superficial bottom inflow guiding the water to the drains is suggested. Another way of eliminating organic material from the bottom on a regular basis is through a suction device attached to a hose connected to a suction pump and operated either by a diver or from outside the pool with a long pole. It is important to eliminate all organic material as fast as possible from the system as they can be the cause water contamination. Water drainage tubes should have at least 20 cm in diameter and should be covered by a protective grid that allows safety to the animals. Only tube valves should be chosen where the valve disk opens completely. Butterfly valves are not suitable.

2.4.2 Mechanical Filtration

Pre Filter – Screen Filter

Even if most of the actual facilities work with only one pre-filter it is recommended to use a Dual Pre Filtration system. In the first step large particles (i.e. leaves, faeces, branches) are being removed from the system. In the second step, smaller particles (but to big for the sand filters) are being eliminated.

Screen filters are the most common pre Filter system used in manatee pools. They use some sort of fine mesh material (normally made in stainless steel) through which effluent passes while the suspended solids are retained on the screen.
There are various types of screen filters. Wire screens are being used in some facilities and consist of a static hillside metal screen with mesh material. There are no moving parts and solid particles descend over the mesh to a channel whereas the water goes through the mesh into the next filtration step.
The most common used screen filter is the drum filter. With this configuration waters enters the open end of a rotating drum and passes through a screen attached to the circumference of the drum. The size of the particles the drum can filter depends on the filter plateholes. A high pressure jet of water from the outside of the drum washes the solids off the screen. The biggest advantage of drum filters compared to other screen filters is the large surface area of the drum.

Figure 13: Drum filter (courtesy Zoo Nuremberg)

As already mentioned at the beginning a Dual Pre Filtration system would be the best solution in order to get most of the large particles out of the system before entering into the sand filters. One possibility would be for example a combination of wire screen filter first and then a drum filter.

Sand Filter

Once the water passed though the prefiltration process eliminating big sized particles, the pumps send the water to the sand filters. The most common types are Rapid Gravity Filters (flow rates of 2.5 -5.0 m^3/m^2 per hour) or Sand Pressure Filters (depending of the type with flow rates from 10 – 12 m^3/m^2 per hour). If well designed, for example by keeping a reasonable ratio of surface area of the sand filter vs. pool volume, all types of sand filters provide good service. They consist of closed vessels, made either in steel or glass fibre, containing a body of sand held above a supporting bottom drain assembly. Normally water is pumped into the vessel and passes the sand which strains and retains particulate matter. As soon as the sand gets clogged the pressure across the filters increases. When the prescribed level for cleaning the filter bed is reached, the filter flow is reversed and the dirt and water used for the cleaning operation is diverted to waste; sometimes, compressed air is used to "fluidise" the filter bed during the back wash cycle to ensure total cleaning. When the water washing the filter bed appears run clean, the system flow is returned to normal direction and continues to filter the pool's water.

Figure 14: Steel filter (courtesy Zoo Nuremberg)

In the last years steel filters have been replaced by glass fibre filters as they have a better resistance to corrosion and the system normally consists of many filters (instead of only one big steel filter). This has the advantage that the filtration can run 24 hours, 365 days, even if one of the filters is being backwashed.

Sometimes, filtration aids are added to sand filters, like for example the aluminium- based chemicals. This chemical aids the precipitating (coagulating) of small particles within the water making it easier for them to be trapped by the filter bed. Additionally it is recommended to add iron-based chemicals to eliminate phosphates in order to control algal blooms. However, as with any chemical addition,

Figure 15: Protein skimmer (courtesy Zoo Nuremberg)

testing and control is import as its accumulation within the pool water can cause eye and mucus membrane irritation. The effectiveness of all these chemicals is always pH dependent. Therefore continuous control of pH values is required. Filter design and construction is also a key factor that can affect the functionality of the chemical reaction. Normally it is best to have many smaller sand filters instead of only one big filter.

Protein Skimmer

A protein skimmer is another mechanical system to remove organic matter and to reduce nitrogen compounds often used in facilities holding manatees. The pool water is mixed with air and pumped into the reaction chamber. Proteins and solids become attached to the air bubbles and flow upward and concentrate in a foam that is being eliminated from the system. The foam generators on these systems can also incorporate ozone that increases the oxidation of organic material. Caution has to be taken to ensure that when using fresh water the protein skimmer has to be designed for this medium. The main difference of protein skimmers for salt and fresh water lies in the size difference of the bubbles. For fresh water this bubbles should be much smaller.

2.4.3 BIOLOGICAL FILTRATION

Nitrification

Nitrification is a biological process by which nitrogenous organic compounds are mineralised (converted into inorganic nitrogen). Most nitrogen in organic matter is mineralized to ammonia (referred as NH_4-N), which in high concentrations is toxic to most aquatic mammals. In practice, this biological filtration is used principally to remove biological waste products and control bacteria.

The principals involved in nitrification are the deliberate culturing of species of aerobic bacteria known for their ability to convert ammonia to nitrite (*Nitrosomonas sp.*) and nitrite to the nitrate (*Nitrobacter sp.*). As the conversion of ammonia to nitrate is normally given by providing optimum environments (temperature and oxygen) the removal of nitrate is more complicated. This process is normally achieved in special chambers (Bioreactors) located after particulate filtration, which containing media such as stones or plastic balls (Bio Balls) that offer a large surface area for the bacteria to grow on. Bioreactors have one big disadvantage, they are very space consuming. Large areas are needed in order to accommodate the Bioreactor.

2.4.4 STERILIZATION

Chlorination

Chlorine is a good bactericide, but it has been criticized because it may destroy beneficial microflora and inactive antimicrobial substances secreted by the skin of many species of aquatic mammals resulting in some cases in severe skin infections (Geraci, et. al., 1986).

Even if chlorine is one of the most popular disinfection method used in Zoos it is not recommended for manatees because of two main reasons:

1. Normally manatees are being shown in mixed species exhibits, in most cases socialized with fishes that do not tolerate chlorine.
2. It is unknown if chlorinated water has a prejudicial effect on manatees that do drink fresh water.

Chlorine is a chemical agent used for the elimination of microorganisms and algae. Opposed to ozone and UV sterilisation chlorine is used as an oxidising agent in the bulk-fluid and has a prolonged time of action. Chlorine based products are inexpensive and has a strong antimicrobial property. However they present important disadvantages in that they form products with lower oxidation potential (chloramines) as well as mutagenic and carcinogenic byproducts, which are dangerous if not removed from the system by means of chemical processes or frequent water renewal.

Chlorine is a good bactericide, but it has been criticized because it may destroy beneficial microflora and inactive antimicrobial substances secreted by the skin of many species of aquatic mammals resulting in some cases in severe skin infections (Geraci, et. al., 1986).

Ozone

Ozone (O^3) is an allotrope of Oxygen and is being used as point-contact oxidising and bactericide agent having also effects in removing water turbidity. Four processes are involved: ozone gas generation, gas-to-liquid absorption, contact time for reaction and finally the removal of ozone residual (Summerfeldt and Hochheimer, 1997). Ozone is harmful to aquatic organisms at very low levels. Summerfeldt and Hochheimer (1997) report a lethal minimum concentration of 0.01mg/l for fishes. Ozone is very unstable and has to be produced on site; it is generally mixed with treated water in a special reaction chamber before the water is returned to the pool. It is important that all ozone is removed before the treated water returns to the pool as it can be very noxious and dangerous (Dineley, 1998).

Ozone has many advantages, like for example the high potential to kill both bacteria and virus, and has beside that the capacity of clarifying the water, avoiding in this way the need of using coagulation chemicals such as aluminium sulphate (Dineley, 1998).

However ozone has disadvantages as well, because of his point-contact oxidising nature, it does not leave any form of disinfectant residual in the pool water. This can lead to uncontrolled algae and bacteria growth on pool surfaces. But in order to cope with this situation chlorine in small dosages can be used in conjunction with ozonation. Furthermore ozone is dangerous to humans and difficult to measure correctly.

It is suggested that in totally closed circuit water systems the use of ozone sterilisation is in favour, although more expensive, it is more effective and safer for the animals (Joustra, 2003).

Keaffaber and Coston (1998), designed a Redox Balance Model for manatees and other marine-mammal and fish aquarium systems. It was found that three distinct Redox environments are required to achieve nitrogen management, Sterilization, and overall animal health in recirculating systems. The redox potential (Eh) values at the Living Seas were observed to be +850, +500, and +200 mV in the aeration tower (after ozone aeration), the manatee pool, and the denitrification basin, respectively. Note: Approximately 200 mV must be subtracted from these values to determine the redox potential observed in the field using standard state Pt redox electrodes. These three values differ by approximately 300 mV, and they represent three very different water-quality environments. They range from a highly oxidizing, disinfecting environment to a nitratereducing, anoxic environment.

The use of ozone is compatible with the use of protein skimmers.

UV light disinfection

Ultra-Violet (UV) light irradiation, is a physical process that is quite widely used, mainly for small volumes of water, but is very efficient also for large systems. This system works because UV light penetrates an organism´s cell walls and disrupts the cell´s genetic material, making reproduction impossible. As with ozone UV Radiation is a point-contact process. Efficiency depends on the time of contact, hence the speed of water flow is crucial. Turbidity limits the efficiency of UV. The main advantage of using UV, rather than chemical disinfection, include: absence of toxic byproducts, has no volatile compound (VOC) emissions or toxic air emissions, does not require storage of dangerous material and does not change the chemical quality of the water, and for this reason, it is the safest among the current techniques available, both for animals and humans.

2.4.5 FRESH / SEA WATER

Manatees in the wild are found in fresh, brackish and salt water. Even if only few data is available on the ability of Antillean Manatees to osmoregulate and maintain water balance, it is known that manatees need access to fresh water when kept in salt water. Fresh water can be provided either by a hose or by eating food items that are high in water content (e.g., lettuce which contains approx. 94% water). Antillean Manatees have been held in Zoos in either fresh water or seawater (with freshwater drinking supply) without consequences. Observations made in captive manatees kept in freshwater suggest that they get a great deal of water from the food they eat and from drinking large quantities of water.

2.4.6 Summary

There is hardly a consensus of what constitutes a properly designed Life Support System for manatees. There is no single solution in order to assure a good water quality. It is rather a combination of different techniques that can be the most efficient solution. But as research in this area is active and new techniques evolve, the right decision is always a matter of evaluating the last developments in accordance with size requirements, budgetary constraints, design and animal species composition of the planed enclosure. Based on actual developments and findings but also on the experience gained in the last years it is recommended to consider the following steps when designing a manatee exhibit:

1. Overall design should be made by experts of life support systems for animal management
2. It should allow constant water circulation through the entire pool
3. Solid material on surface and the bottom should be removed either by providing an optimum water flow or by designing a pool bottom with a tilt to the bottoms drains.
4. Drain design should take into account safety for the animals
5. A dual pre-filtration System is recommended.
6. 100% whole water volume should flow through sand filters.
7. It should be guided afterwards to appropriate protein skimmers.
8. Finally we recommend to use ozone or UV for disinfection: one can also predict to utilize both system in different % of the water volume or in line; if ozone is utilized, carbon filtration could follow before the water re-entering the tank.
9. Minimum flow rate should cover 100% of the total volume with a turn over of maximum 2 hours.

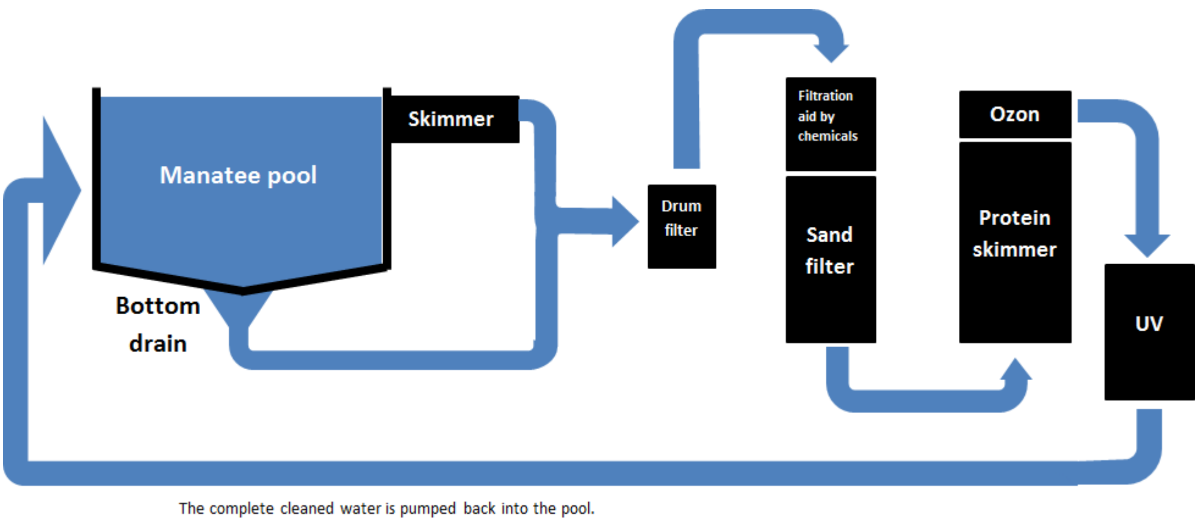

Figure 16: Outline of a Life Support System.

2.5 WATER PARAMETERS

In order to evaluate the water quality it is recommended to monitor the following parameters:

- Temperature
- Bacteria (e.g. coliforms)
- pH
- Nitrate (NO_3^-)
- Nitrite (NO_2^-)
- Turbidity
- Ammonia (NH_3)
- Phosphate
- Dissolved oxygen
- Ozone
- Redox Potential
- Salinity
- Chlorine derivates (even when no chlorine but ozone only is utilized)

The coliform bacteria count of the primary enclosure pool shall not exceed 1000 MPN (most probable number) per 100 ml of water.

The specific water quality tolerances and requirements for marine mammals in general vary considerably, but typical maintenance ranges for water in the tank are shown in Table 2. These are general numbers for marine mammals, specific maintenance ranges for manatees are not known for all parameters.

Table 2: Maintenance ranges for water in the tank (DEFRA, 2004)

	Marine	**Freshwater**
NH_3	<0,05 ppm	<0,1 ppm
Nitrite	<0,1 ppm	
Nitrate	<20 ppm	
0^2	>6 mg/L	>6 mg/L
pH	7,9-8,4	6,5-9
Redox	340+/-20 mV	
Salinity	27,5-32 ppt	5-9 gr/lt sometimes used in therapy
Temperature – tropical	25 - 30 ºC	22-28ºC

It is important that the parameters are checked on a regular basis. In table 3 it is said if water samples should be taken and tested for the parameters on a weekly or daily basis.

Table 3: Schedule check parameters (USDA, 2001*a*)

Parameter:	**Check:**
Coliform	Weekly
Salinity	Weekly
pH	Daily
Chemical additives	Daily
Nitrate (NO_3^-)	Daily
Nitrite (NO_2^-)	Daily
Ammonia (NH_3)	Daily
Temperature	Daily
Turbidity	Daily

2.6 FEEDING

Proper nutrition is indispensable for keeping animals in a good condition. Nutrition can have a large effect on basic health as well as physical and mental development, reproduction, behaviour and survival. Individual nutritional requirements include proteins for structural and hormonal development, a good balance of all vitamins for general health and wellbeing and minerals for metabolic function, skeletal structure and many other functions.

2.6.1 DIET AND FOOD QUANTITY

Sirenians are the only group of fully aquatic herbivorous mammals. Due to their low metabolic rate and to the fact that manatees are primarily herbivores, the food requirements of a manatee are 5-20 Kcal/kg/day (CCAC, 1984). Adult males and non-lactating females consume about 4-8% of their body weight/day; calves consume about 15% of body weight/day; and lactating females eat about 10-13% of their body weight/day (Best, 1981). To fulfil these requirements, a manatee in a Zoo should eat on average 4 to 7 % of their body weight. For an adult manatee with a weight of 500 kg this would be 20-35 kg per animal.

A general guideline is to provide a diet that incorporates 70–85% leafy green vegetables (e.g. romaine and iceberg lettuce, cabbage) or ryegrass, or hydroponic wheat, oats, and/or sprouts; 10 to 20% dried forage such as timothy or alfalfa hay (filtration must be upgraded to handle dried matter), about 5% of other vegetables and fruits (carrots, yams, apples, bananas, etc.), and various commercially available dry pelleted feeds (monkey chow, elephant flakes, etc.). The specific vitamin and mineral requirements for manatees are unknown. (Dierauf and Gulland, 2001). Even if manatees are primarily herbivores, they occasionally and sometimes even like to eat invertebrate and fish meat.

In summary, the dominant food items in the diet of a manatee are:

- Salad or ryegrass (approx. 80% of the daily diet)
- Regarding the remaining items, especially in the case of potatoes, carrots, beet manatees would prefer them, when they are precooked.

A daily feeding ration for an adult manatee (500 kg weight) can contain (Diet Zoo Nuremberg):

1. Salad or ryegrass. Grass for spring - summer months (between 12 to 17 kg per animal) and salad for autumn – winter months (between 18 – 25 kg per animal).

2. Diverse vegetables – raw (tomato, paprika, cucumber, zucchini, celery, aubergine, Chinese cabbage, cauliflower, chicory, carrots) 5 l bucket per animal.

3. Diverse vegetables – cooked (fodder beet, beet root, carrot, fennel, kohlrabi, pumpkin) – 3.6 l bucket per animal. These vegetables are being cooked in tab water with approximately 1 teaspoon of salt.

4. Corn – approximately 0.4 l per animal.

5. Wheaten – approximately 0.25 l per animal.

6. Manatee "suet balls" (wheaten bran, oat flakes, calves grain, insect shred, 1 spoon of sea salt, 1 banana and water) – approximately 0.5 kg per animal.

7. Pellets (for example ALTROMIN 6024) – approximately 125 gr.

8. Roughage (branches, hay, sweet corn rods and other items). Preferred Branches: willow (*Salix sp.*), maple (*Acer sp.*), beech (*Fagus sp.*), banana and palm branches. Because of toxicity avoid branches of false acacia (*Robinia pseudoacacia*). Also avoid overuse of hay because it can result in dehydration.

Figure 17: Branches

Another important note is the addition of salt to the diet in case the manatees are being kept in fresh water systems. In case the animals live in salt water fresh water has to be offered. Feeding diets deficient in salt may not show immediate symptoms, but on the long-term can lead to health problems associated with hyponatremia.

In general terms, a rough and practical approach, based on experience, is to test different food items in order to discover what your animals really like most. Manatees do not only consume a wide variety of food items, they also show individual and seasonal differences in their food preferences. It might be the case that they have to get habituated to some food. Therefore just test in order to find out what they really like!

2.6.2 Nutrient values

Zoobiologists, veterinarians and keepers recognize the crucial role of nutrition in the maintenance of good health. However the total nutrient content of what a manatee eats per day can only be defined by evaluating the nutrient value of the different food items that belongs to the manatee diet.
See table 4 for the nutrient values of the main food items given to manatees.

Table 4: Nutritional Value of Feeds and Manatee milk (© Walsh & Bossart, 1999)

Feed	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)	Calories (per 100g)
Romaine lettuce	95.6	0.9	0.04	0.6	2.9	16
Green leaf lettuce	94.8	1.1	0.1	0.8	3.2	18
Sea grass	87.0	1.8	0.2	2.5	8.5	43
Wheat (hydroponic)	87.0	2.8	0.5	0.2	9.7	60
Water lettuce	93.0	1.2	0.7	1.5	3.5	25
Water hyacinth	93.0	0.8	0.2	1.4	4.6	23
Timothy (75%) and alfalfa (25%)	6.0	8.1	2.2	7.0	76.7	354
Manatee milk*	75.0	7.5	15.5	1.0	1.0	189

* milk from midlactatation

2.6.3 Feeding method

Observations made in the wild shows that free-ranging manatees graze 6 to 8 hours/day. Based on this data it might be reasonable to feed the animals many times per day. Smaller quantities of food given more frequently during the day will permit normal grazing behaviour and help prevent filtration systems from being overwhelmed.

Manatees are normally fed three to five times per day within a timeframe of up to 8 hours. In most cases the animals get a portion of leafy green vegetables or ryegrass in the morning, once it is consumed new salad or ryegrass is being offered. All other items, like potatoes, cucumber, celery, beet, etc. are being fed distributed through the day.

For practical reasons it is recommended to offer the leafy green vegetables or ryegrass in some sort of big net bags (see Fig. 21). Another way of offering this leafy foot is by placing them in a hoop (see Fig. 19). In using these last two feeding methods a smaller quantity of salad or grass will end up in the filtration system. All other items like sweet pepper, tomatoes, zucchini, aubergine and others can be mounted on a wire assembled like a garland (see Fig. 20). In order to force the animal to use all parts of the exhibit smaller items can be scattered in the pool.

Figure 18: Hoop with salad (courtesy Zoo Nuremberg)

Figure 19: Garlands with different vegetables (left) and salad (right) (courtesy Zoo Nuremberg)

Figure 20: Net bags with ryegrass (left) and hoop with salad (right) (courtesy Zoo Nuremberg)

Especially prepared manatee "suet balls" (see Fig. 22) can be used as reinforcement for habituation and training purposes. As already mentioned it is a question of testing and patience to find out what your individual animal like most. It was shown for example that for training purposes some Zoos use liquid reinforcement (pineapple juice), fish / shell meat, pieces of fruit and / or toast bread.

Finally, it is the responsibility of the keepers to monitor feeding behaviour as daily food intake may have to be adjusted in order to prevent obesity and maintain behavioural motivation for training purposes, while at the same time satisfying the animals' nutritional needs.

Figure 21: Suet balls (courtesy Zoo Nuremberg)

2.7 Social behaviour – Group size and composition

Manatees are not overly gregarious and normally live in loosely associated groups. The strongest social bond is formed by mothers and calves and last for approx. two years. In Zoos, manatees can be kept in different group structures: male groups, female groups and breeding groups composed by adult females and males. Manatees are promiscuous, with multiple males mating with individual females. The experience has shown that many combinations of males and females, either together or separated in one group is possible. Even many sexually mature males with adult females are possible and based on the assumption that manatees are sperm competitors perhaps the best option to encourage reproduction. Therefore multi pool enclosures as it is recommended in the case of other aquatic mammals species, is not compulsory. However some Zoos did have social problems, when mother and their newborn calve were disturbed by other manatees. In this case barriers installed in the main pool can be used in order to temporarily separate the group.

Regarding group size it was shown that even high numbers of individuals per exhibition, independently of the composition by gender, do not cause visible social problems.

2.8 Behavioural Management

2.8.1 Training

Manatees have been trained using operant conditioning techniques for different purposes. One area that demonstrated the training capabilities of manatees is the investigation of diverse sensory modalities. For example hearing thresholds of manatees was first investigated using operant conditioning techniques via a Go-NoGo discrimination procedure (see chapter Sensory Systems). Another series of experiments where manatees had to be trained using a simultaneous discrimination procedure refers to the sensory role of the perioral bristles around the mouth of the manatee. Using the same procedure other researchers could even investigate colour vision in manatees.

While training procedures with manatees for research projects are a common method in order to answer many scientific questions regarding their biology, only in the last years Zoos are investing time and resources to develop training programs for their animals. One example is gating. Moving one animal from one pool to another is easily accomplished by training. Especially training medical behaviours should be a standard routine within animal management strategies. Up to now only few manatees in Zoos are being trained for medical checks. Given the fact that manatees showed to be capable of learning complicated tasks, as it was demonstrated in various scientific studies, it is surprising that these training methodologies are not being used by Zoos as a normal husbandry routine. This routine should allow keepers and veterinarians to take measurements for biometry, body inspection, collect fecal samples, allow blood sampling, body temperature measurements and ultrasonographic exams.

2.8.2 ENRICHMENT

In general terms enrichment comes in many shapes and forms (Hosey, *et. al*., 2009). In the case of manatees only food based enrichment seems to be applicable. It is important to clarify that any change in an animals´ life or their environment can be considered as enrichment. Though, it is important to make sure that these changes should confer some sort of benefit to the animal without negative ramifications. Therefore enrichment procedures have to be constantly evaluated regarding their effectiveness.

As manatees spend a large proportion of their day searching for and eating food, the way how food is offered, seems to be one reasonable way of enrichment. Some feeding enrichments aims to prolong the feeding experience, by making the acquisition of food more difficult. As already described in the previous section (Feeding Methods) salad or ryegrass net bags is one possibility to keep the manatee busy manipulating food. In general terms as manatees are known to manipulate food items, either with the flippers or the mouth, any food based enrichment should consider this trait. Scattering food items may be another way of enrichment, in this way foraging can be stimulated.

Other forms of enrichment like sensory or social enrichment are also worth to mention even if there is no evidence that manatees may profit from these measurements. As manatees are equipped with many sensory capabilities (see section sensory systems), enrichment using for example tactile or auditory information may provide a form of sensory enrichment. Regarding social enrichment the easiest form is given trough the various types of interactions within the members of the group. Furthermore manatees are well known to inhabit enclosures with other species, in so called mixed species exhibits. Certainly, combining manatees with various animal species in the same enclosure has primarily practical and educational reasons. So far there are no clear indications that manatees profit from the interaction with other species.

In zoos manatees share their enclosure with fish like guppy's, arapaimas, catfish and pacus; turtles; rays, like freshwater stingrays; capybaras and birds. Caution has to be taken when choosing for example the right fishes, in Zoo Parc de Beauval the introduction of the threespot leporinus (*Leporinus friderici)* resulted to be detrimental for manatees as fish bites caused serious skin lesions to them. Large terrapins and stingrays must also be watched, particularly when approached by calves.

2.9 BREEDING / REPRODUCTION

The mating herd is the most obvious potential breeding behaviour in the wild, whilst in zoos the female is being pursued by one or more males. Mating has not been observed frequently. Pre-copulatory activity in manatees is intensive and prolonged. Mating lasts for a few days (3-4) in which males go off food almost totally and the females copulate with all the males continuously. During mating, both male and female lie horizontally in the water in a ventral to ventral position. Gestation length is thought to be around 12-14 months and on 2 occasione it duration has been measured exactly for 347 and 357 days (so probably slightly less then 1 year). Normally a single calf is being born with a weight of average 20 kg (18 - 25 kg) and a length of 1,20 m (based on data from the EEP population). There are cases of twins, but normally only one individual will survive.

The calves nurse from one of the two nipples located in each axilla behind the flippers and starts within 48 hours after birth Normally the calf and the mother float horizontally and the calf swims in the direction of the nipple. The only assistance of the mother is given by the fact that she swims slowly holding her flipper out of the way. Normally weaning occurs after the calf is 2 years old, but start eating solid food (for example lettuce) when 40 days old. In zoos cows have been observed to nurse their own calf and other much larger calves that are not her own.

Usually females giving birth do not show clear behaviours that can be used to predict this event. A massive enlargement and protrusion of the vulva appears approximately 4 to 5 months before birth. Some observations revealed that some variables and subjective changes such as distension of the abdomen and visible opening of the vulva appear to indicate imminent parturition together with the presence of white mucous. Ultrasonography is the safest way to control pregnancy. With repetitive training and certain degree of skill, gained by applying this technique on a regular basis, ultrasonography is an excellent way to determine if the animal is pregnant, if the foetuses are viable and what the gestational age is. If the animal is trained for blood sampling the IMMULITE®´s progesterone assay seems to be a valid and highly sensitive and specific tool for pregnancy determination in manatees, particularly during early and mid-gestation. Changes in serum progesterone concentrations throughout manatee gestation could be measured, showing the highest concentrations occurring during early pregnancy (Tripp, et al., 2008).

The birth usually takes place within a group and in most cases there is no problem in keeping mother and calf together with all other members of the group. However in some cases, the mother-calf bond may be disturbed by other manatees. In this case a temporal separation of the both animals from the group is recommended.

Regarding reproductive parameter referred to the Manatee population in European zoos, the youngest female at first reproduction (age are as of parturition) was 3 years and 7 months old. Oldest mother was 30 years old. Shortest interbirth interval was 442 days. In one Zoo (Nuremberg) one female had 13 offspring during her whole life. There is no evident birth seasonality, however there is a peak of births between August and October. Regarding males the youngest male to reproduce (age are as of conception) was 4 years old.

Once the calf is born a thorough protocol of observation should put in place in order to follow its process and anticipate possible progresses by intense observation of the dyad. Consistent suckling usually starts within the first 2 days and its progress is relatively fast but regular.
Respiration rate is one of the important parameter to make sure that the animal is ok or if surffering from possible problem, not only respistaroy nut also due to lack of energy to thrive.

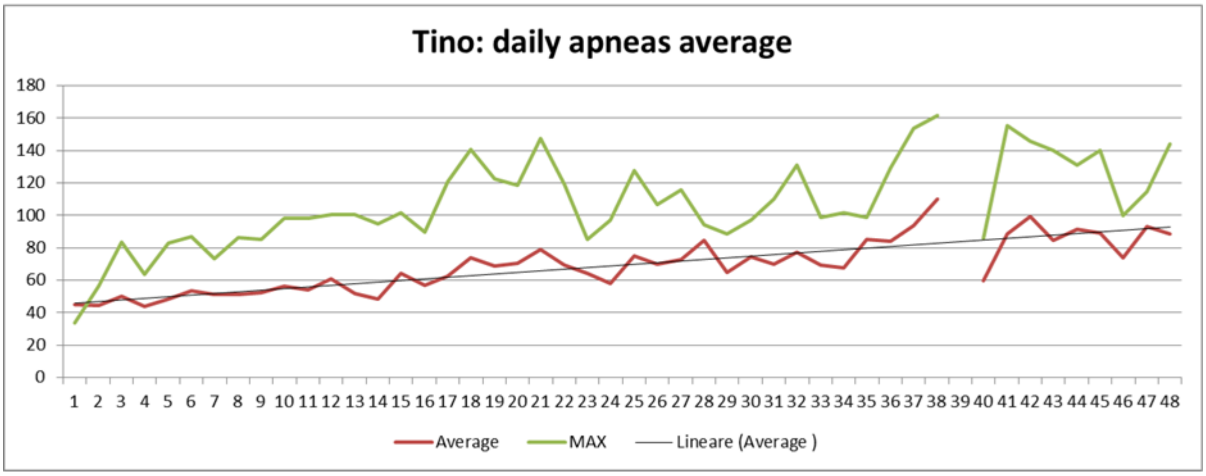

Figure 22: The fgraph shows a normal trend of average apnoeas in the first 50 days of life taken 4 times a day.

Regarding neonatal mortality until 2014 55 births have been recorded within the EEP population. 41 of this calves lived more than one year (73%), seven died before reaching one month of age (13%), only one died before reaching the age of one year (2%) and finally 7 were recorded as premature or stillborn (13%).

Hand rearing

Hand rearing a calf can became an option in cases where the mother does not take care of the calf. Causes for that can be: the mother has not enough milk or she just rejects the calf or does not have the ability to engage the calf and the rearing procedure. Several occasions of hand rearing a manatee have been registered, generally with a positive result. At this point it is important to remark that even if the decision has been made to hand rear the calf, the calf should still be kept with the mother. In some Zoos it was shown that a hand reared calf started nursing after 39 and 40 days and no extra bottle- feeding was necessary.

If the decision was taken to hand rear the calf it is recommended to follow the following protocol.

Hand rearing protocol

Main objectives:

- get the calf stabilized on a formula
- have them feed from the side of the pool.

Practical Information

1. Water temperature: as thermoregulation is critical for manatees try to increase the temperature to approx. 29 – 32°C. Low water temperature will prevent good weight gain and will decrease the calf immunity.

2. Weight control: try to weight your animal regularly in order to see if it is gaining weight. Once the animal starts losing weight they become prone to secondary infectious diseases.

3. Blood values: calves should be sampled at least once a week to monitor their hydration status, electrolyte disturbance and to evaluate their overall condition. Initially, it is important to monitor blood glucose levels. Manatee calves take a little time to adjust and utilize a fat source so it would be recommendable to add simple sugar (dextrose) until the gluconeogenesis pathway is efficiently established.
Blood from calves are easy to collect: the ventral tail vein is easily accessible turning the calves on its back or just lifting the tail. The needle can be entered at 90° on the mid line.

4. Monitor stool: one of the most common problems associated with the use of Formula is constipation. Beside that they seem to be prone to gastrointestinal issues ranging from diarrhea to constipation to *pneumatosis intestinalis* where air bubbles form in the wall of the intestines. Therefore it is important to monitor fecal output.
5. Behaviour: watch for depression or lethargy associated perhaps with hypoglycemia. Monitor respiratory rate (normal respiratory rate: 3-5 breaths in 5 minutes during regular activity). If the calf arches his back it could be a sign of constipation or intestinal pain, for example by gas accumulation.

Formula:

Analysis of manatee milk has revealed that taurine is a major amino acid and that short- and medium-chained fatty acids are abundant. With this information, it was determined that a mixture of Esbilac (or Milk Matrix 33/40) with 16 ml of either soybean or canola oil was well tolerated.

The Formula is probably the single most crucial element and gastrointestinal problems are very common. These animals generally don't do well on diets that contain large amounts of carbohydrate (human, sheep, goat, cow replacement formulas). The carbohydrates produce gas, leading to distension and colic, with difficulty submerging and stomach gas, colitis, necrosis etc. If optimal formula is not available as soon as you start hand-rearing, add lactase tablet to the milk to limit the lactose intake. If gas colic are observed or suspected, simethicone at 20 to 40 mg/feeding is useful. X-ray can help on the evaluation of gas amount. If decrease appetite due to colic is observed, it's advised to go back to clear fluids for 12 to 48 hours and then increase the amount of milk slowly. If constipation or reflux are observed metoclopramide (and not cisapride) at 2,5 mg is useful.

Generally when you start hand rearing the calf, check a baseline blood sample and resolve any dehydration, electrolyte disturbance, and hypoglycemia with replacement fluids. Human products like Pedialyte or calf replacement fluids work well for this. Use rehydration fluids independently of the milk formula. Mix standard water to energy fluids.

Start them on half strength formula. Measure by volume (not weight) and use 1 part powder to 2 part water then progress to 1 to 1 and finally 1.5 to 1. Use the 1:2 for a few days and then if the animal is doing well on that concentration gradually increase it to 1:1. To help the transition from clear rehydration fluid to ½ strength formula, add 50% Dextrose (makes formula sweet and keep glucose level high); dose at 1 to 2 mg/100ml of formula; wean off gradually after 1 to 2 weeks. By now the animal will be gaining weight at the rate of a ¼ to ½ a kg per day. Again, if the animal is handling the formula well gradually increase again to 1.5 Milk Matrix to 1 of water.

The formula of choice is Zoologic Milk Matrix 33/40 by Pet-Ag, Inc.; a closely related product is Esbilac puppy replacer also by Pet-Ag.

- *Zoologic Milk Matrix 33/40 or Esbilac 500gr*
- *Water 1000ml*
- *Taurine 250mg/l*
- *formulaB- complex 1 tablet/l formula*
- *Canola oil 4 to 16 ml/100g formula*

Feeding, especially at the beginning might take some minutes, therefore in order to keep a constant temperature of the formula an insulation of the bottle (i.e. neoprene) is recommended.

Hygienic control
For each feeding new freshly prepared formula should be used. Bottles and nipples should be cleaned and disinfected after every feeding.

Energy requirements and daily requirements

An approximation of energy requirements for orphan manatee is 50 - 80 kcal/kg for maintenance plus growth.

So if the weight of the calf is about 20 kg they need approx. 800-1000 ml Formula/day. Once the calf is suckling regularly 800 is probably achievable but 1 Liter per day would be pushing it. Often the caloric content can be increased by two ways: slowly go up to 1.5MM : 1 cup of water and/or start to add some oil; usually canola oil, again starting low 1-2ml/100mls formula and increase it to maybe 4 ml per 100 ml formula.
Calves nursing well on a bottle may gain 0.3 to 0.5 kg of weight per day.

Feeding frequency

Five to seven times a day (every two-three hours, for example starting at 7.00 AM and last feeding at 22:00 PM). Calculate volume of each feeding according to the energy requirements (see above).

Delivery method

Use a baby bottle with a lamb or calf nipple. The nipple used for nursing is a cow/bovine nipple that is soft (boiled in water to soften) but does not collapse when the calf sucks (Figure 23 and 24).

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Figure 23: Bottle-feeding of a manatee calf


Figure 24: Bottle with long nipple

Several techniques are successful to encourage an animal to accept and suck the nipple of a bottle. Often, an animal will suck on fingers. Once this has begun, fingers can be removed and the nipple of the bottle inserted into the animal ' s mouth.

It is important that when the calf latches on and sucks that it is rewarded with a fair amount of milk so the hole or cut in the nipple is fairly large that lets milk flow pretty easily. Further, cut the bovine nipple down to fit the ring of a plastic human baby bottle preferable those human baby bottles that are tilted or slanted at about a 45 degree angle. This angle facilitates nursing baby manatee while they are flat on their bellies in a horizontal position. Try to feed the calf in a horizontal position, at the beginning it might be necessary to take the animal out of the water but with time try to feed the animal in the water. If you start outside of the water, wet towel on the head of the animal can help calm it down. Remember you will have to bottle feed this animal for months so try to find the most comfortable way for both calf AND team.

Figure 25 and 26 show the device and feeding method developed with success by Odense Zoo. This device was introduced some days after birth and certainly the biggest advantage is that the keeper does not have to catch the animal.

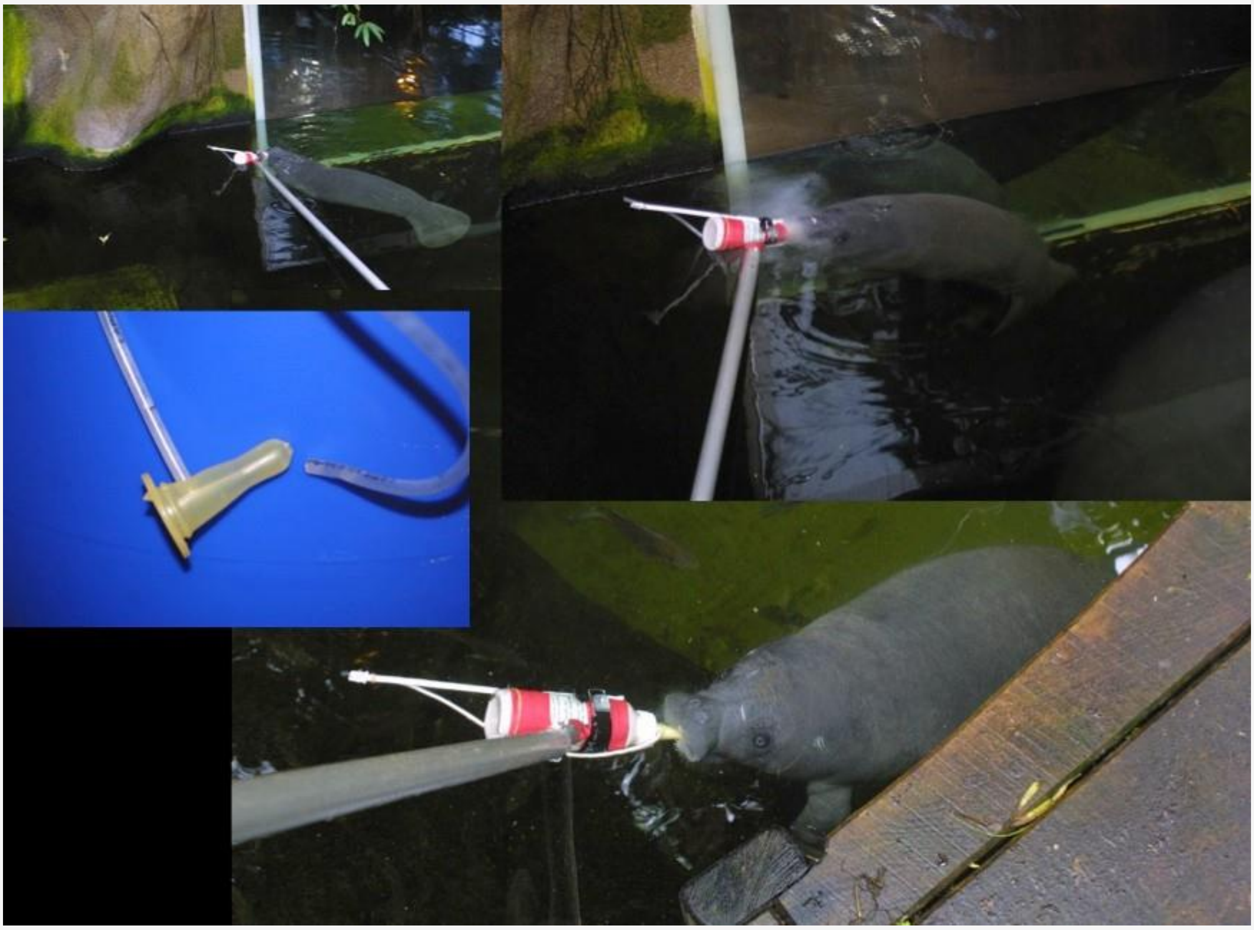


Figure 25: Bottle-feeding device (© Bjarne Klausen, Zoo Odense)

Figure 26: Bottle-feeding device (Photos © Bjarne Klausen)

Caregivers should use similar nursing techniques to encourage the calf to accept the bottle more rapidly.
Avoid the use of stomach tubes (only in cases when the animal is not doing well).

When calves are left with the mother, training the mother to accept temporary retrieval of the calf for feeding is very important in order to avoid any stress in the dyad. The mother can also be constantly behaviourally reinforced with food while stationing during calf feeding.

Nursery log

Try to set up nursery logs (Fig. 27) in order to record formula offered, amount offered, amount consumed, stool condition, and the animals overall behaviour at each feeding.

Species:
Common Name:
House Name:

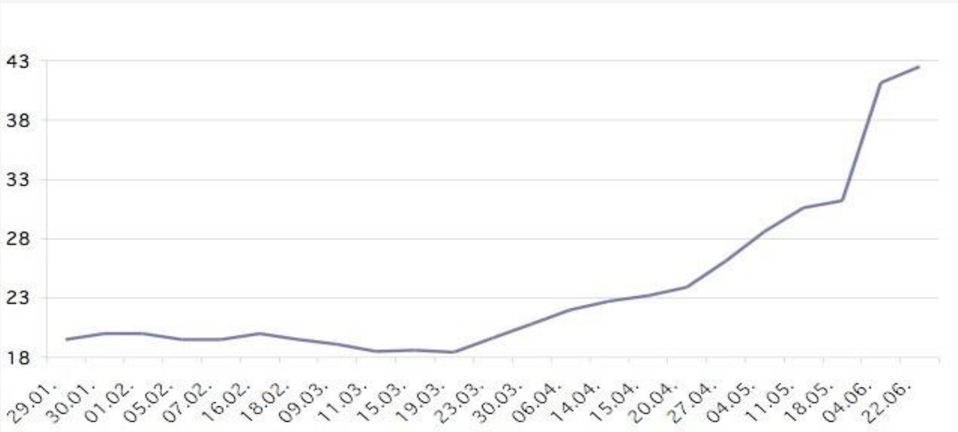
Date	Time	Formula Concentration	Amount offered	Body weight	Stool	Water temperature	Behaviour	Breathing rate	Comments

Microbiological assessment of the fecal samples of the calf is very important from the beginning and integration of faeces from the mother in the formula has also been used to stimulate the intestinal activity.

Gaining weight

Normally when bottle-feeding calves with formula a weight gain of 250 – 500 gr. per day should be expected. However do not be frightened if that's not the case. It is advisory to follow the instructions of the protocol and if everything fits then patience is recommended. As shown in the next graph (Figure 28) one hand reared manatee (Herbert) at Zoo Nuremberg did not gain weight for the first 70 days. Today he is doing fine and does not show any sign of problems. Even if at the beginning he did not grow in a normal way the graph clearly show that weight, even if it is important, should not be the crucial factor in evaluating the fitness of a hand reared calf. As shown in the next two graphs Figure 28 and 29, hand reared manatees did not gain weight for the first two months, but afterwards. Today all three are doing fine and do not show any problems.

Body weight



Date

Figure 27: Example of growth of a hand-reared manatee (Herbert, Zoo Nuremberg, 2009)

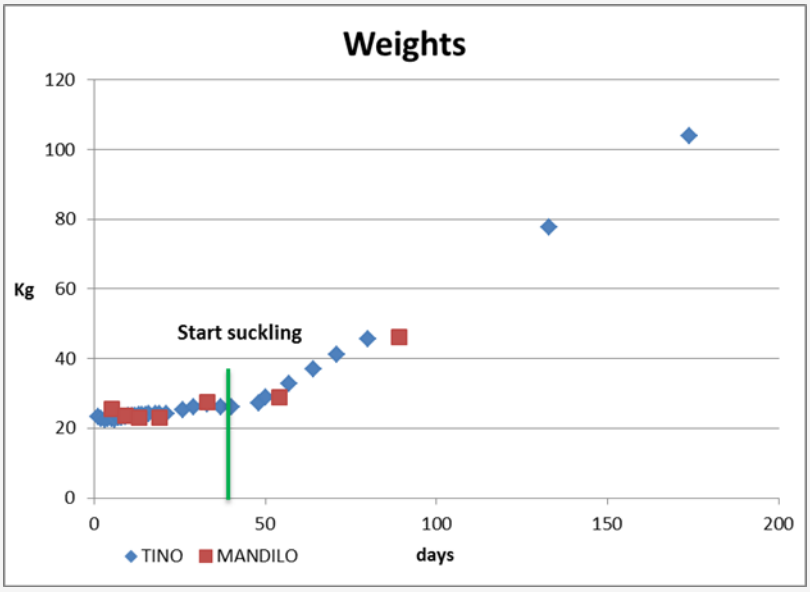


Figure 28: Tino and Mandilo two calves that were first bottle-fed, but after more than two months started suckling from the mother normally

This protocol is based on information provided by:
- Dr. Dave Murphy (former Vet at Lowry Park Zoo)
- Dr. Baptiste Mulot (Zooparc de Beauval)
- Dr. Katrin Baumgartner (Zoo Nuremberg)
- Dr. Bjarne Klausen (Odense)
- Dr. Joseph Gaspard (Mote Marine Laboratory & Aquarium)
- Dr. Fernanda Löffler Niemeyer Attademo (Med. Vet. CMA/ICMBio, Itamaraca, Brazil)
- Claudia Gili, DVM,PhD (Acquario di Genova)

2.10 Veterinary care

Due to the fact that manatees in zoos do not show health problems quite frequently their overall health status might be misleading mainly because regular checkups, including for example blood analysis, are quite difficult to perform. Like it is done with other species the diagnostic work starts with the observation of the animal, physical examination, skin inspection, body condition, feeding records and when possible, a history.

As with many other aquatic mammals training is an important prerequisite to handle manatees in case it is needed. Therefore it is highly recommended to invest time and resources in order to have a good control of the animals via training.

In acute health situations (but also recommended for regular checkups) blood analysis has to be carried out. As comparison values of complete blood cell count (CBC) and serum chemistry profiles from 12 healthy Florida manatees (there are no data available on Antillean manatees) is shown in Table 5.

Blood is taken from the interosseous space between radius and ulna (see Fig. 29). In order to avoid contamination the venipuncture site has to bee properly disinfected by surgically scrubbing the area for at least two minutes. Faecal samples are easy to obtain. Urine can be obtained with a lot of patience by applying pressure on the abdomen anterior to the vulva in females and posterior to the genital opening in males. Ultrasonography can be used in case of suspected pregnancy.

Figure 29: Left: Blood sampling (© v. Fersen); Right: Blood sampling location (Walsh & Bossart, 1999)

Table 5: Values for Complete Blood Cell Count (CBC) and Serum Chemistry Profiles of 12 Healthy Florida Manatees (Walsh & Bossart, 1999).

Test	**Value**
CBC	
Hemoglobin (g/dl)	10.3-12.0
Hematocrit (%)	33-38
Hematocrit/PCV (%)	32-40
RBC ($10^5/mm^3$)	2.41-3.06
MCV (fl)	121-135
MCH (pg)	37-43
MCHC (g/dl)	30-33
RDW (%)	16.0-21.5
Platelets ($10^3/mm^3$)	261-634
MPV (%)	5.7-7.3
NRBC/100 WBC	1-2
WBC (mm^3)	4000-11,70(
Bands (%)	0
Neutrophils (%)	25-64
Lymphocytes (%)	21-77
Monocytes (%)	0-18
Eosinophils (%)	0
Basophils (%)	0-1
Serum Chemistry	
Glucose (mg/dl)	56-117
BUN (mg/dl)	6.4-16
Creatinine (mg/dl)	0.4-2.1
Bilirubin total (mg/dl)	0-0.1
Cholesterol (mg/dl)	107-328
Triglycerides (mg/dl)	34-138
Total protein (g/dl)	6.8-7.3
Albumin (g/dl)	3.8-5.3
Globulin (g/dl)	1.7-3.2
Alkaline phosphate (U/	64-183
ALT (U/L)	6-30
AST (U/L)	5-28
GGT (U/L)	39-64
CK II (U/L)	79-302
LD (U/L)	94-372
Calcium (mg/dl)	10.1-12.2
Phosphorus (mg/dl)	10.1-12.2
Sodium (mEq/L)	142-157
Potassium (mEq/L)	4.2-6.6
Chloride (mEq/L)	90-103
CO_2 (mEq/L)	14-43
Iron (µg/dl)	59-199

PVC	packed cell volume
RBC	red blood cells
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
RDW	red blood cell distribution width
MPV	mononuclear phagocyte volume
NRBC	nucleated red blood cells
WBC	white blood cells
BUN	blood urea nitrogen
ALT	alanine aminotransferase
AST	aspartate aminotransferase
GGT	gamma-glutamyltransferase
CK	creatine kinase
LD	lactate dehydrogenase

Medications can be administered orally mixed in some preferred food items. Antibiotics given orally may result in loss of intestinal flora and diarrhea and therefore are not recommended. Because of this negative side-effects most antibiotics are given in the caudal epaxial muscle. It is important to disinfect the injection site properly. In cases where manatees have to be rehydrated stomach intubation can be used. Also nutritional supplementation is administered by gastric tubes.

Critically sick animals should be kept in a pool with good water quality, have to be provided with a fresh water source (not pool water) to decrease bacterial compromise and water temperatures of approximately 29,4 °C.

A comprehensive and more detailed description of veterinary medicine procedures as well as diagnostics techniques, medication / treatment procedures and diseases was written by Walsh and Bossart (1999) and published in Fowler and Miller, Volume 4, Current Therapy.

As the EEP manatee population is quite small in numbers of individuals, all information of the animals counts. Therefore it is imperative to examine every dead animal (see Necropsy Forms in the attachment, Appendix 2, 3, and 4). Causes for neonatal mortality has to be analyzed in more detail. Also the lost of the foetus, either premature or fully developed has to be investigated. In this regard it is important to mention that in the past they have been cases of recurring dermatitis in some animals. In two cases (Joop & Luna) the animals died and postmortem examination revealed a possible autoimmune dermatosis (Leistra, et al., 2003). As some individuals are genetically susceptible to developing autoimmune diseases it is advisable to pay more attention to this occurrence within the population. As a matter of fact in the last 16 years the EEP population registered 29 births, 12 died soon after birth. Eight of this 12 death calves are genetically related to Joop or Luna, the manatees that died due to a suspected autoimmune disease.

2.11 HANDLING

2.11.1 IDENTIFICATION

Even if the Manatee EEP population is quite small and therefore only few animals per Zoo are being kept, it is recommended to implant tag transponders (PIT Passive Integrated Transponder) once the animal is restrained. The tag is surgically placed subdermally into the cutaneous space of the fat layer over the right or left shoulder region using a cut-down procedure and a modified injection syringe (Dierauf & Gulland, 2001). Make sure to disinfect properly the injection site. Furthermore manatees can be identified quite easily on the basis of scars, pigment variations, size and other irregularities.

2.11.2 Catching / Restraining

Manatees can cause important trauma if handled inappropriately and by un-experienced people. Proper protocol and contingency plans must be in place for employers safety regulation.
The easiest and safest way to handle manatees is by applying operant conditioning training methods. Through training manatees can become habituated to the keepers and to different husbandry routines. Gating and general body inspection is easily accomplished once the animal was trained for that. If the animal is trained to swim from one pool to another, the manatee can be guided to swim into the medical pool, where the animal can be treated.
In cases where the animals are not trained or not prepared to follow training signals from the keeper others methods should be used. It is important to keep in mind that manatees can be very strong and quite forceful when keepers need to capture the animal. The use of divers in the water and nets in order to catch the animal are not recommended as manatees can get entangled in the net or the animal may harm the diver. It is safer to use strong net-based barriers or panels to direct the animal towards the medical pool, providing in this way a secure separation between animal and human. Another proven way to impel a manatee in a certain direction is by “spooking” him with a quick blast of air from a scuba regulator in the muzzle. Once the animal is in the medical pool the first step is to drain the pool and laying the animal on the false bottom (if available) or placing the manatee on a stretcher in low water.
Restrain the animal by fixing first and foremost the tail. Usually this can be achieved by placing a piece of thick foam on the paddle and the weight of two or three people. Also try to fix the whole body as manatees can roll on their sides. In case of prolonged treatments out of the water manatees should be sprayed with water to avoid skin from drying.

For transport purposes the following loading steps are suggested (depending on the facility):

- separate the animal (e.g. med pool)
- prepare the pool to be drained
- lift the crate alongside the animal
- pull crate back (in shallow area of the pool)
- drain poll during the procedure
- make sure that the stretcher is below the animal
- tighten ropes in order to secure the animal
- lift stretcher with animal and place both on a big foam
- take blood, hair and other samples
- weight the animal
- lift stretcher and animal into the crate
- fill in the crate with water from the manatee pool (approx. 26°C)

Figure 30: Stretcher used for transport (courtesy Zoo Nuremberg)

2.11.3 TRANSPORTATION

Manatees have to be transported in special crates (see Fig. 31). In order to have a soft bottom in the crate on which the manatee will be transported the crate is lined with a thick foam (at least 30 cm) that covers the bottom and the sides of the box. Usually a resistant plastic foil impervious to water covers the whole foam. Usually the crate is only open at the top, but new crates do have a drop end opening allowing unloading the animal much easier (see Fig. 32). The crate should be slightly larger in length and with that the manatee, in order to enable the animal to make normal postural adjustments. Usually crate dimensions should be adapted to the size of the animal. Usual crates have the following dimensions: height 160 cm / width 130 cm / length 350 cm.

Figure 31: Manatee crate (courtesy Zoo Nuremberg)

Figure 32: Unloading a manatee from a crate with a drop end opening (courtesy Zoo Nuremberg)

Wet transports are favoured, it means that once the animal is in the crate pool water should be added to a level that covers 2/3 of the whole animal. Dry transports can be an option but only for short distances.

Airplane / Ground

For air transports use aircraft cargos where the cabin can be pressurized at an altitude equivalent to 8.000 – 10.000 feet and a constant temperature can be kept. Ground transportation is done by using a tempered truck. In either case a veterinarian and an experienced keeper should escort the transport.

In general terms safe transports should follow some experienced based recommendations protocols, the most important requirements are listed below.

Manatee Transport Protocol

Important requirements and samples needed

- Before the transport the water values of both facilities (donating and receiving) should be compared

- The medical records, blood values and other laboratory data of the animal should be send to the receiver

- Emergency drugs should be taken on the transport

- Body weight and morphometric measurements should be taken (see below)

- Pictures of the animal (details like the tail) should be taken

- Faecal samples should be taken before and during the transport: part of them can be use for parasitology, bacteriology and mycology; part of it should be frozen for further investigations (like cortisol, progesterone). All samples need to be labeled with the name of the animal, the date and the time

- Saliva samples (Salivette Sarstedt®) should be taken (if possible) before and during the transport: all of them need to be centrifuged and frozen, all samples need to be labeled with the name of the animal, the date and the time: these can be used for cortisol determination (and be indicators of stress during the transport)

- Blood samples should be taken: EDTA (2: 1 for the checkup – 1 for genetic evaluations), Plasma (1 for the checkup) and Serum (2: 1 for the checkup – 1 frozen for further evaluations): in females progesterone should always be measured, in males testosterone

- Skin samples/swabs should be taken: a mycological examination can be performed

- Hair samples should be taken and stored: these can be interesting for cortisol and genetic investigations

During the transport the following records should be taken on a regular basis (eg. every 90 Minutes):

- Air and water temperature
- Breathing rate
- Salivary swaps (if possible)
- Position of the animal

Items that must be taken along the transport:

- thermometer
- portable light
- transport protocol
- foam mats
- knife
- sponges
- water cans
- feeding protocol for the animal
- CITES
- Emergency drugs

Table 6: Manatee transport emergency medication list.

Indication		Medication	Concentration	Volume per 500 kg body weight
Agitation	short term *or:* long term	Midazolam Diazepam =Valium®	15 mg/3ml 10 mg/2ml	4 ml i.m. 6,6 ml i.m.
Antidote	*(sedation is too deep)*	Flumazenil =Anexate®	0,1 mg/ml	4 ml i.m. for Midazolam 6,6 ml for Diazepam
Acute dyspnea, pulmonary emphysema, asthma		Bronchoparat® =Theophyllin	20mg/ml	initial 4 ml then: per hour 0,8 ml i.m
Lung edema		Furosemid 2mg/kg =Lasix®	10mg/ml	100 ml i.m. BID
Apnea		Doxapram	20 mg/ml	10 ml i.m.
Shock	*first:* *after that:*	adrenaline 1 : 1000 Solu-Decortin H®	1 mg/ml 1000 mg/10 ml	→ dilute with 9 ml aqua; inject slowly up to 25 ml i.m. 30 ml i.m.
Reanimation, bradycardia	 *respectively:*	Atropin Adrenalin 1 : 1000	100 mg/10ml 1 mg/ml	2 ml i.m. → dilute with 9 ml Aqua; inject carefully up to 25 ml i.m
Antibiotics		Rocephin Biklin Enrofloxacin	2 g dissolved in 10 ml 500 mg/2ml 100 mg/ml 1,25 mg/kg body weight	55 ml SID 28 ml SID 625 mg = 6,25 ml SID

APPENDIX 1: ETHOGRAM OF MANATEES (GOMES, ET AL., 2008)

Major categories	
Category	Definition
Resting	Subject motionless with eyes closed, either at the surface, with head and tail pointing downwards, or at the bottom of the pool, making only unconscious movements to the surface to breathe at regular intervals (normally 2 to 5 min, but up to 25 min).
Exploring	Subject moves slowly in search of food, water, or other individuals.
Drinking water	Subject ingests fresh water from pool-side source.
Circular movement	Subject moves around the enclosure continuously in a circular fashion. May include rotation around the body's own axis.
Interaction with object	Subject investigates inanimate objects present in the enclosure, including freshwater pipes, gates, and pool edge.
Ingesting faeces	Subject ingests pieces of faeces submerged or floating on the surface of the water.

Miscellaneous categories	
Category (code in Table 3)	Definition
Embrace (Ab)	Subject uses one or both pectoral flippers to grasp other individual's back or side.
Hit with flipper (Bnp)	Subject hits other individual forcefully with one of its pectoral flippers (adapted from Rosas, 1994).
Head butt (Ca)	Subject pushes other individual with movements of the head (adapted from Linhares et al., 2001).
Copulation (Cp)	Male subject exposes penis and penetrates genitals of female individual.
Corkscrew movement (Dp)	Subject moves forward while rotating its body around the longitudinal axis.
Walk on bottom (Cm)	Subject moves along the bottom of the pool supported by alternating movements of the extremities of its pectoral flippers.
Push away (Emp)	Subject pushes other individual with its body or a pectoral flipper, impeding its approximation to a food item or another individual (adapted from Linhares et al., 2001).
Expose penis (EP)	Male subject approaches another individual (male or female) and embraces it with a pectoral flipper as it exposes its penis.
Interact with person (Ip)	Subject advances to the edge of the pool to investigate approaching human, normally remaining at the edge until the human moves away, and occasionally mounting the edge of the pool.
Autogroom (LC)	Subject rubs itself against a surface of the pool or uses its pectoral flippers to remove pieces of food or excess skin from its body.
Suckle (Ma)	Subject maintains its snout in contact with the base of the pectoral flipper of other individual (Rosas, 1994).
Diving with tail exposed (Me)	Subject dives under the water holding its tail flipper above the surface of the water.
Mount (Mo)	Male subject positions himself over the back of a female and, normally, exposes its penis (Rosas, 1994).
Corkscrew (Mp)	Subject rotates its body around the longitudinal axis without forward movement.
Tail slap (Ra)	Subject repels other individual through horizontal or vertical movements of the tail flipper, which may or may not make contact with the body of the other individual (adapted from Linhares et al., 2001).
Attempted copulation (Tc)	Male subject attempts to slot his body together ventrally with that of other individual (male or female) without successful penetration (adapted from Linhares et al., 2001).
Touch snout (Tf)	Subject touches back, head, or snout of other subject with its own snout.

APPENDIX 2: HEALTH STATUS REPORT

Survey about the health status in Antillean Manatees / Manatee EEP

Responsible Person

Name:

Mail Address:

Zoo:

Manatee Name:

Studbook Nr.:

Male ☐ **Female** ☐

---

1) General information

Size:

weight: estimated

weighted

Do you train your manatee?

no

yes, for

---

2) Medical report

Please add reports, results and photos you took as that can help to create standard values and can be used in further studies.

2.1. Reproductive activity and issues

For Females

Age of first estrus:

Describe sexual behaviour:

1

Knowi Pregi encies?

no

yes/ nuniler/ years | normal ☐ / /
resorgrion / /
0!Oltic›n ☐ / /
dyslocia ☐ / /
srillbirth / /

For Males

Age of first se:Dual behaviour:

Descrile sexual !:e haViOUr

Call sired ?

No ☐ unknown

Yes successtul

unsuccessful f2DDrtiOn en.)

For females ai d males

Mating observed

no

yes • frequent

Amy causes of reproductive issues hnown?

no

infection • viral !J2C£9ñi3I ☐ parasitic ☐ fung 21

endo‹rinological

nutrition

environment

partner

dysfunction - Please nane organ:

cuncer

unsolved

If a report ‹an not l:›e added, please descrile issues shortly:

Samples collected:

- ☐ no
- ☐ blood ☐ faeces ☐ urine ☐ saliva
- ☐ biopsy ☐ hair ☐ swabs ☐ placenta ☐ foetus

Diagnostic:

- ☐ bloodwork ☐ serology ☐ clinical chemistry ☐ endocrinology
- ☐ virology ☐ bacteriology ☐ mycology ☐ cytology
- ☐ histology ☐ x-rays ☐ ultrasound

☐ others: ________

Treatment: ________

Problem solved?

- ☐ yes, with treatment
- ☐ yes, on its own
- ☐ no, recurrent
- ☐ no, chronic

2.2. Behavioural/ neurological

- ☐ no problems ☐ aggression ☐ stereotypy ☐ disorientation
- ☐ lethargy ☐ abnormal movement ☐ coordination disorder

other ________

Cause:

- ☐ nutrition
- ☐ endocrinological
- ☐ environment
- ☐ central nervous, specify ________
- ☐ infection → ☐ viral ☐ bacterial ☐ parasitic ☐ fungal
- ☐ immunological
- ☐ unsolved

3

If a report can not be added, please describe course of disease shortly:

Samples collected:

- [ ] no
- [ ] blood
- [ ] faeces
- [ ] urine
- [ ] saliva
- [ ] biopsy
- [ ] Hair
- [ ] swabs

Diagnostic:

- [ ] bloodwork
- [ ] serology
- [ ] clinical chemistry
- [ ] endocrinology
- [ ] virology
- [ ] bacteriology
- [ ] mycology
- [ ] cytology
- [ ] histology
- [ ] x-rays
- [ ] ultrasound

- [ ] others:

Treatment:

Problem solved?

- [ ] yes, with treatment
- [ ] yes, on its own
- [ ] no, recurrent
- [ ] no, chronic

2.4. Skin

- [ ] no problems
- [ ] wounds
- [ ] efflorescences, please describe (for dissemination, feel free to use draft at the end of the survey or add photos)

5

Causes:

infeccion • viral ☐ !J2C£0ÑÑI ☐ parasitic ☐ fung2l

trauma

environment

nutririon

inimunologiC2l

endcrinological

cancer

unsolved

If i3 repoft can not In added, please dese rile course of disease shortI¿

Samples collected:

no

Island ☐ faeces urine ☐ Si3lÍVi3

biopsy ☐ h2ÍF swabs

Diagnostic:

bloodwork ☐ serology ☐ cliGichenWst¿ ☐ endwñnology

virobgy ☐ !Ji3C£RÑOlCg/ ☐ myoology r#ology

histology ☐ x-rays ☐ ulóasound

☐ others:

Treaorient:

Problen solved?

yes, mil treatnient

ARS, C€\ ÍtS OWD

no, recurrenr

no, chrcnic

2.5. M isc iloskeletal

no pro!›Ienis ☐ paralysis QIJnOfTfli3I moran ☐ i3bnormal posture

i3rhrophia

☐ others:

6

ise:

fracrure

trauma

environ menu

neural d'/sfuncton ☐ cenml ☐ periphera l

nutrition' nialdigestion

infecrÖn • viral ☐ bacterial ☐ parasitic ☐ fung al

cancer

inimunological

endocrinological

unsolved

If i3 m rt can not In added, please desc rile course of disease shortly:

Samples collected:

☐ no ☐

☐ lilood ☐ faeces ☐ urine ☐ saliva

☐ hĺopsy ☐ h2ir ☐ Swabs

Diagnostic:

☐ IJlccÓwork ☐ serology ☐ clinical chemistry ☐ endocrinolcgy

☐ viroDgy ☐ ♭acmriology ☐ mycology ☐ cytology

☐ histology ☐ x-rays ☐ ulDasound

orhers:

Treaonent:

Problen1 solved?

yes, wil treatment

yes, on its oym

no, recurrenr

no, chronic

6 Res i o

no pro!›lenis

infection • virul !J2CÎPÑ2I parasitic fung2I

inimunological

environmenr

dysMnction - Please ni3nie organ.

cancer

unsolved

If 2 repoft can not In 2dded, please des rile course of disease shortly

Samples collected:

no

hlo‹ l faeces urine 5i3I ï'7i3

biopsy hi3ir Swubs

Di*0nostic:

bloodwork serology clinical chemistry endocrinology

virobgy !Ji3CLRÑOlOCjy mycology cytology

histology x-rays ultrasound

orhers:

Treasnent:

Problen sotved?

yes, 11 treatment

y 5, 0fl it5 OWPI

no, recurrenr

no, chronic

8

Œhers

no

renal/ urinary

in munological

CgFdÏ6V3SCUî3T

other:

If a report can not be added, please describe course of disease shortly:

Samples collected:

no			
hlood	faeces	urine	saliva
biopsy	Mir	swabs	

Diagnostic:

bloodwork	ology	clinical chensisry	endccrinology
virology	bacteriology	mycology	cytology
histology	x-rays	ultrasound	

others:

Tr+mment:

Problem solved?

yes, vrii treatnent

ÿ9S, Œ\ its OWD

no, recunenr

no, chrcnic

9

2.7. Health problems in species kept in the same enclosure:

☐ no
☐

Recse describe or aMch report

Did you preserve any samples yDu ever took from these animals?

☐ no
☐ BDod ☐ faeces ☐ urine ☐ saliva
☐ Bbpsy ☐ hair ☐ swabs

2.8. Morphornetric Measurements

A— straight length:

B — curvilinear length:

C – axillary girth:

D — um'uilical giioi:

E — peduncle girth:

F - Blubl:er Depth (Ultrasound) prin ai1Iy at the dorsal site aligned with the umhilicus:

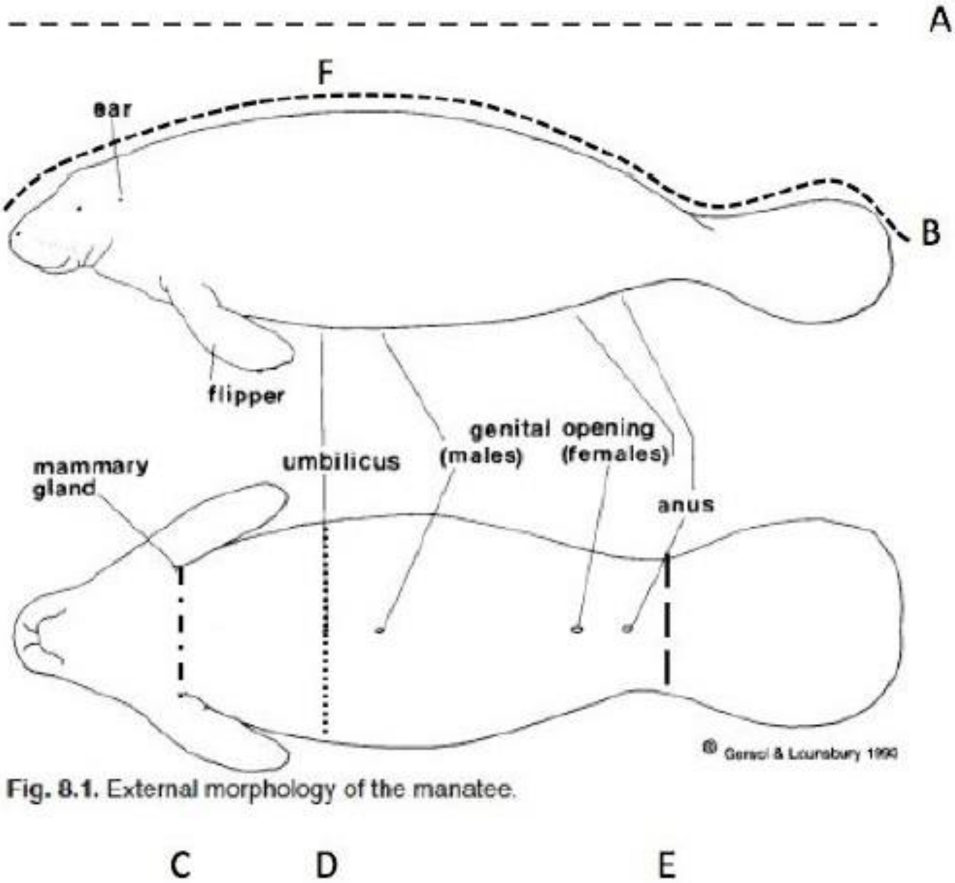


Fig. 8.1. External morphology of the manatee.

APPENDIX 3: NECROPSY REPORT FOR ADULTS AND NEONATES

NECROPSY FORM FOR adult Antillean Manatees

1) General information

Animal ID:

Date/ time of death: /
(dd-mm-yyyy/ hh:mm or estimated time)

Date/ time of necropsy: /
(dd-mm-yyyy/ hh:mm)

Location of necropsy:

Contact of pathologist:

Performed by: **Assisted by:**

2) Preliminary report

Type of death: ☐ euthanasia ☐ peracute ☐ acute health issues ☐ chronic health issues

Please add detailed report, results and photos if documented or use following.

If not send yet, we would be pleased if you could add a whole medical report including results and photos of the animal as that can help to raise standard values and can be used for further studies.

Behavior:

Clinical symptoms:

Treatments:

Diagnostics:

1

3) Examination

Sex:	☐	male	☐	temple		
Level of nutrition:	☐	poor	☐	moderate	☐	good
State of autolys is :	☐	fresh /rnild	☐	moderate	☐	advanced

3.1. Body measurements

Body weight (kg):

Body length (cm):

Body girth (cm):

3.2. Marphorrietric Measurements

A— straight length:

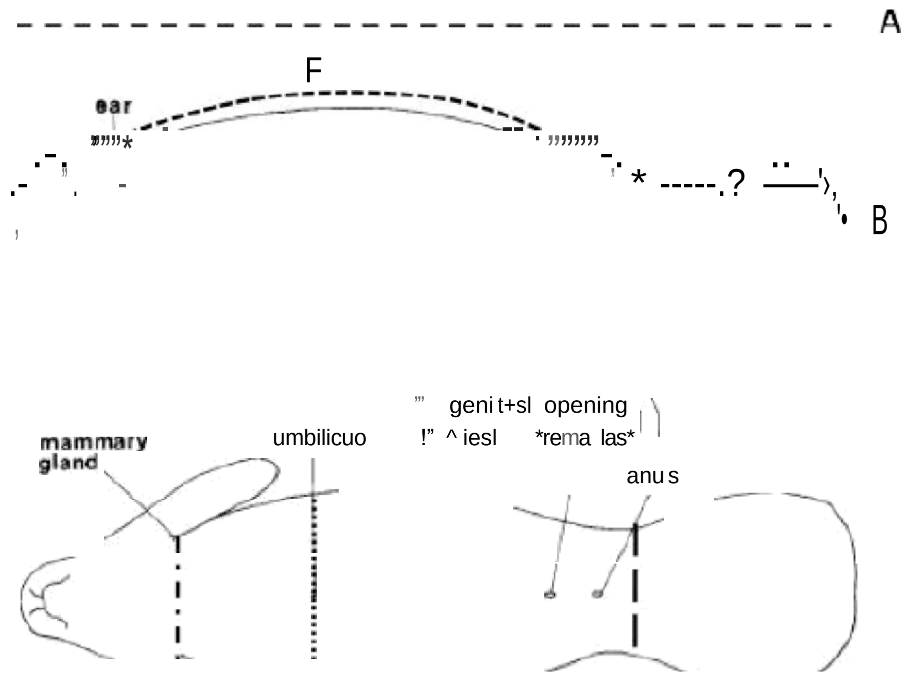
B — curvilinear length:

L: —axillary girth.

D — umbilical girth:

E — peduncle girth:

F - Blubber Liepth (Ultrasound) prin ally at the d‹xsal site aligned with the umbilicus:



rig. *D.4* . ExtemaJ or gho og1' of I hc r-ianol c-c

C D E

3.3. Gross examination of arctan systems

Please note checklist for nee‹le‹l samples on the sheet ' IMfoi tJJ‹ition al›orit studies on autoiiJ fJJuiJe ‹lisease in Antillean MaiJateas'".

'Ae would Ice gl;3d, if ',au could add or send photos you took and results front hiStopcthDlogy etc.

3.3.1. Respiratory

- [ ] not examined
- [ ] no abnomialities detected
- [ ] abnormal ,

description

Heasuremeria:

Samples collected for:

☐ ND,'oecause
☐ Histopathology Virology ☐ Bacteriology
☐ omen
☐ Autoimrriune studies (University of Leipzig)
Tissues onount:

3.3.2. Diqestive

☐ not examined
☐ no abrircmalities detected
☐ abnormal, description

Hecsuremeria:

Samples collected for:

ND, because
Histopzthology ☐ Virology ☐ Bacteriology
☐ ofhem
☐ Autoimmune studies (University of Leipzig)
Tissue/ amount.

3.3.3. Urinary

- [ ] not ex mined
- [ ] no abnornialities detected
- [ ] abrorT tzl . deSCriptiOrl

Heasuremena:

Samples collected:

- [ ] No, hecause
- [ ] HisBpathology
- [ ] VIFOIO
- [ ] Bacterioloqy
- [ ] omem

- [ ] Autoimmune studies (Ltniversity of Leipzig)
Tissue/ amount.

3.3.I Genital

- [ ] not examined
- [ ] no abnornialities detected
- [ ] abrormal , deSCriptiOrl

Heasuremena:

Samples collected for:

- [ ] No, hecause
- [ ] Hisopokoogy
- [ ] Virology
- [ ] Bacteriolopy
- [ ] Dthem

☐ Autoimmune studies (University of Leipzig)
T!ssue/ annum:

3.3.5. Cardiovascular

☐ not examined
☐ no abriomialities detected
☐ 2bf\0fTI1i3,I description

Neasuren ents:

Samples collected:
☐ No, because
☐ Histaputhalogy ☐ Virology ☐ Bacteriology
☐ Omen
☐ Autoimmune studies (University of Leipzig)
Tissue/ annum:

3.3.& Nervous, braiM and senses (incl. Eyes. vibrisses

☐ not examined
☐ no ahriomialities detected
☐ ahriomial .
description

Heasuremena:

Samples collected for:

- [ ] No, because
- [ ] Histopatholc•gy
- [ ] VIFOIO@
- [ ] Bacteriology
- [ ] Dthei

- [ ] Autaimmune studies (University of Leipzig)
Tissue/ arnount:

3.3.7. Musculoskeletal

- [ ] nDt exarnined
- [ ] nD abnornialities detected
- [ ] abr›ofTÏlZl . deSCriptiOls

Heasuremena:

Samples collected for:

- [ ] No, because
- [ ] Histopathology
- [ ] Virology
- [ ] Bacteriology
- [ ] Dthec

- [ ] Autaimmune studies (University of Leipzig)
Tissue/ arnount:

7

3.3.8. IMteqixrient incl. hair

- not examined
- no i3bnOlTnalities detected
- abnormal , description

Measurements:

Samples collected for:

No, because

Histopathology ☐ Virology ☐ Bacteriology

☐ Other

☐ Autoimmune studies (University of Leipzig)
Tissue/ i3nount:

3.3.9. Lymphoid

- not examined
- no 2bnOlTl1alities detected
- abnormal , description

Measurements:

Samples collected for:

- [ ] No, l›ecouse
- [ ] Histopcthology
- [ ] Virology
- [ ] Bacteriology

- [ ] Other

- [ ] Autoimmune studies (University of Leipzig)

Tissue/ i3nounr:

1.3.10. End rine

- [ ] not examined
- [ ] no abnDlTl1Qlities dete-ered
- [ ] ;3L¥T0fTl1:,3l description

Neasurenients:

Samples collected for:

No, l›ecause

Histopnthology
- [ ] \1rology
- [ ] Bacteriology

- [ ] Other

- [ ] Autoimmune studies (University of Leipzig)

Tissue/ ¡3nounr:

4) Necrapsy results

Diagnoses/ Preliminary diagnoses:

Comments:

Analyses pending fDr final dicgnDses:

10

Date of repon: Written by: ________________________________

NECROPSY FORM FOR
Abortion or perinatal death in Antillean Manatees

1) General information

Animal ID:

Date/ time of death: /

Date/ time of necropsy:
'"äd-nan-yyyy/ hh fi n er efitcra fi•d tirrle) (dd-mm-yyyy/ hh:mm)

Location of necropsy:

Contact of pathologist:

Performed by Aslisted by:

2) Preliminary report

Type of deaih: ☐ abortion ☐ stiilbirth ☐ dystocia ☐ neonatal death

Matemal histary

Name/ID:

Age:

Prevîous pregnancies (rfumber, success, any probleme?):

Present pregnancy

Date of conception.

Pregnancy-complications

Treatment:

Last evidence of foetal life (date. time j:

Abortion: D no 0 yes, describe circumstances:

Labour
Onset (date/ timej: /

Rupture of mem bmnes (daté ëme): /

Foetal distress:

- [ ] £J0
- [ ] yes. spe c ill

Presentation:

- [ ] Breech
- [ ] 'v'ertex
- [ ] other

Delivery \date/ time.i. !

Neon.ate

Name/ID:

Behaviour after birth idescribei :

Suckling oMea'ed. [ ] no [ ] yes

Neonatal problems:

Treatment:

3} Examination

Sec:	[ ] male	[ ] female	
Level of nutrition:	[ ] poor	[ ] moderate	[ ] good
State of .autolysis :	[ ] fresh 'mild	[ ] nlodemte	[ ] add an ced

3.1. Body measurements

Bod y we ight (k g j.

Body lenqth 'fens i:

Body girth {cm i:

3.2. Morpliornetric Measurements

A — strai ght length:

B — curvilinear lend th:

C – axillary girth:

D — umbilical girth:

E — peduncle giith.

E - Blubber Depth (Ultrasound) primarily at the dorsal she aligned with the umbilicus:

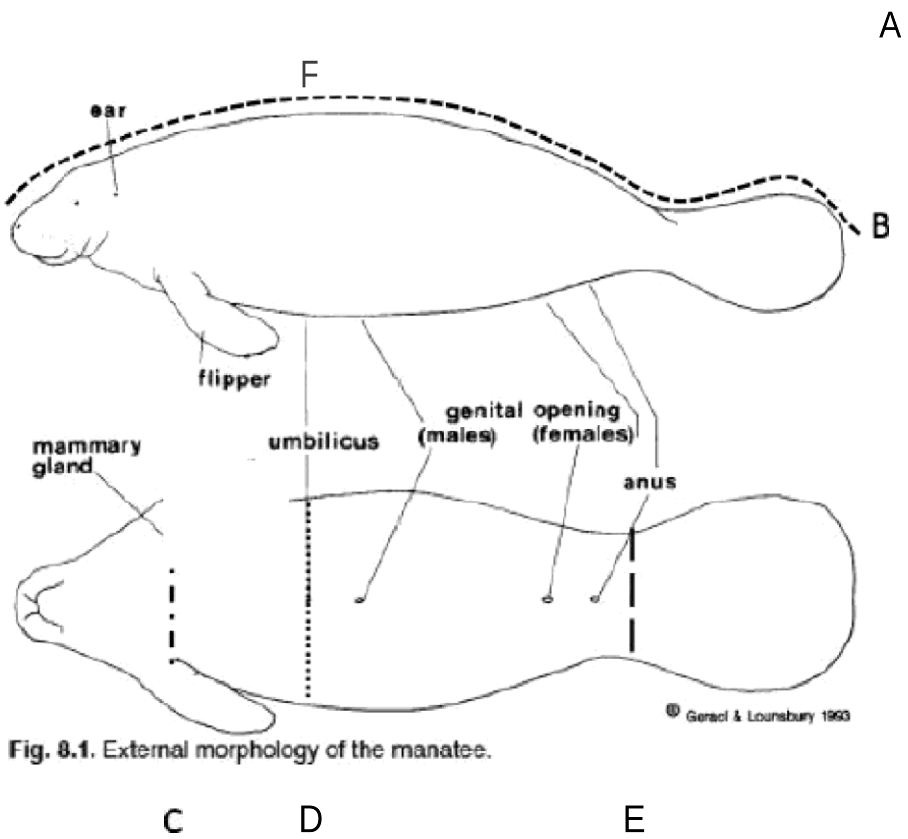


Fig. 8.1. External morphology of the manatee.

3.3. Grass examination of organ systems

Please note checklist for needed samples on tne sheet "Information about studies on autoimmune disease in Antillean Manatees'.

We would be glad, if you could add or send photos you took and results from histopathology etc.

3.3.1. Respiratory

- [ ] not examined
- [ ] no abnormalities detected

☐ abnormal
description

Measurements:

S.nmples collected for:

☐ fJo,becau°e
☐ Histopathology ’v'irology ☐ B«cterioloqy

Others

☐ Autoimmune studie° |University of Leipzigj
Tissues amount.

3.3.2. Digestive

☐ not examined
☐ no abnormalities detected
☐ abnormal. description

Measurements:

S.nmples collected for:

☐ fJo, because
☐ Hi topatho loqy ☐ Virology ☐ Bacterioloqy
☐ Others

☐ Autoinnn1une studies (University of Leipzig:
Tissue/ amount.

3 3 U in

☐ not examined
☐ no abnormalities detecIef
☐ ahnormal . de cripti on

Measurements:

S.amples collected:

☐ £J0, because
☐ Hi°topathology ☐ Virology ☐ Bacterioloqy
☐ Others

☐ Autoinnn1une studies (University of Leipzig:
Tissue/ amount:

3.3.4. Genital

☐ not examined
☐ no abnormalities detected
☐ abnormal, . description

Measurements:

Samples collected for:

- £Jo, bed.ause
- Histopathology
- Virology
- Bacteriology
- Others

- Autoimmune studies {University of Leipzig)
Tissue/ amount.

ñ.3.5. Cardiovascular

- not examined
- no abnormalities detected
- abnormal , description

Measurements:

Samples collected:

- No, because
- Histopatholoqy
- Virology
- Bacteriology
- Others

- Autoimmune studies {Univer ify of Leipzigj
Tissue/ amount:

3.3.6. Nervous, brain and senses (incl. Eyes, vibrisses

- not examined
- no abnormalities detected
- abnormal

description

Measurements:

Samples collected for:

- [ ] tJo, because
- [ ] Histopcthology
- [ ] 'ii FOIO§}
- [ ] Bacterioloqy
- [ ] Others

- [ ] Autoimmune studiez IUniversity of Leipzig)
Tissue/ amaunt.

3.3.7. Musculoskeletal

- [ ] not examined
- [ ] no abnormali4es detected
- [ ] abnormal , descnption

Measurements:

Samples collected for:

- [ ] tJo, because
- [ ] Histopathology
- [ ] Virology
- [ ] Bacterioloqy

☐ Others

☐ Autoimmune studies (University of Leipzig.
Tissue/ amount.

3.3.B. Integurnent incl. hair

☐ not examined
☐ no abnormalities dete cted
☐ abnormal ,
description

Measurements:

Samples collected for:

☐ No, bed.ause
☐ Histopothology ☐ 'ii FOloqy ☐ Bacteriotogy
☐ Others

☐ Autoimmune studies (University of Leipzig)
Tissues amount.

3.3.9. Lymphoid

☐ not examined
☐ no abnormalities dete cted
☐ abnormal . description

Me asurements:

S.nmpl es collected for:

fJo, because
Histopatholog y Virology Bac terioloqy

Olhers

Autoimmune studie° (Univer°iry of Leipzig.i
Tissue/ amount.

1.3.16. End rine

not examined
no abnormalities detected
abnormal . de cripti on

Measurements:

S.nmples collected for:

" fJo, because
[__| Histopathology Virology Bac teriology

Olhers

Autoink n1une studied (Univerñip of Leipzig!
Tissue.* amount.

9

4) Necropsy results

Diagnoses.^ Preliminary diagnoses:

Comments:

Analyses pending for final diagnoses:

Date of report Written by: ______________________________

REFERENCES

Bachteler D.; Dehnhardt G. (1999): Active touch performance in the Antillean Manatee: Evidence for a functional differentiation of facial tactile hairs. In: *Zoology* 102 (1), S. 61–69.

Bauer, Gordon B.; Colbert, Debborah E.; Gaspard, Joseph C. (2010): Learning about Manatees: A Collaborative Program between New College of Florida and Mote Marine Laboratory to Conduct Laboratory Research for Manatee Conservation. In: *International Journal of Comparative Psychology* 23 (4), S. 811–825.

Bauer, Gordon B.; Colbert, Debborah E.; Gaspard, Joseph C. 3rd; Fellner, Wendi (2003): Underwater Visual Acuity of Florida Manatees (Trichechus manatus latirostris). In: *International Journal of Comparative Psychology* 16, S. 130–142.

Best, Robin C. (1981): Foods and feeding habits of wild and captive Sirenia. In: *Mammal Review* 11 (1), S. 3–29.

Blair Irvine, A. (1983): Manatee metabolism and its influence on distribution in Florida. In: *Biological Conservation* 25 (4), S. 315–334.

Bonde, R. K.; O'Shea, T. J.; Beck, C. A. (1983): Manual of procedures for the salvage and necropsy of carcasses of the West Indian Manatee (*Trichechus manatus*) (National Technical Information Service, PB83-255273).

Bullock, Theodore H.; O'Shea, Thomas J.; McClune, Michael C. (1982): Auditory evoked potentials in the West Indian manatee (Sirenia. Trichechus manatus). In: *J. Comp. Physiol.* 148 (4), S. 547–554.

Bossart, G., Ewing, R., Lowe, M., Ghim, S. & Jenson, A. B. (2002) Viral papillomatosis in Florida manatees (Trichechus manatus latirostris). Experimental and Molecular Pathology 72, 37–48.

Caldwell, D.K. & M.C. Caldwell. (1985): Manatees, Trichechus manatus Linnaeus, 1758; Trichechus senegalensis Link, 1795 and Trichechus inunguis (Natterer, 1883), p. 33-66. In: S.H.

Cantanhede, Andrea Martins; Da Silva, Vera Maria Ferreira; Farias, Izeni Pires; Hrbek, Tomas; Lazzarini, Stella Maris; Alves-Gomes, Jose (2005): Phylogeography and population genetics of the endangered Amazonian manatee, Trichechus inunguis Natterer, 1883 (Mammalia, Sirenia). In: *Molecular ecology* 14 (2), S. 401–413.

Cohen, J. L.; Tucker, G. S.; Odell, D. K. (1982): The photoreceptors of the West Indian manatee. In: *Journal of morphology* 173 (2), S. 197–202.

Deutsch, C. J.; Self-Sulivan, C.; Mignucci-Giannoni, A.: Trichechus manatus, West Indian Manatee. Hg. v. IUCN. IUCN.

Deutsch, Charles J. (2000): Winter movements and use of warm-water refugia by radio-tagged West Indian manatees along the Atlantic coast of the United States. Florida Power and Light Company and U.S. Geological Survey.

Deutsch, J.; Reid, James P.; Bonde, Robert K.; Easton, Dean E.; Kochman, Howard I.; Shea, Thomas J O ' (2003): Seasonal Movements, Migratory Behavior, and Site Fidelity of West Indian Manatees along the Atlantic Coast of the United States Author(s): Charles 151, S. 1–77.

Deutsch, C. J., Self-Sulivan, C., & Mignucci-Giannoni, A. (2015): Trichechus manatus, West Indian Manatee. Hg. v. International Union for Conservation of Nature and Natural Resources.

Dierauf L.A. & Gulland, F.M.D. (2001). CRC Handbook of Marine Mammal Medicine. CRC Press, Boca Raton, Florida. ISBN: 0-8493-0839-9

Dineley, J. D. (1998) An Introduction to Basic Water Treatment Protocols for Pinnipeds Exhibits. Paper Presented at Whipsnade Wild Animal Park as part the Association of British Wild Animal Keepers Symposium '98.

Etheridge, Kay; Rathbun, G. B.; Powell, J. A.; Kochman, H. I. (1985): Consumption of Aquatic Plants by the West Indian Manatee. In: *Journal of Aquatic Plant Management* 23, S. 21–25.

Farmer, M., Weber, R. E., Bonaventura, J., Best, R. C. & Domning, D. (1979). Functional properties of hemoglobin and whole blood in an aquatic mammal, the Amazonian manatee, Trichechus inunguis. In: *Comp. Biochem. Physiol.*, 62A, 231-8.

Gallivan, G. J.; Best, R. C. (1986): The Influence of Feeding and Fasting on the Metabolic Rate and Ventilation of the Amazonian Manatee (Trichechus inunguis). In: *Physiological Zoology* 59 (5), S. 552–557.

Gallivan, G. J.; Best, R.C. (1980): Metabolism and respiration of the Amazonian manatee (Trichechus inunguis). In: *Physiol. Zool.* 53: 245053

Garcia-Rodriguez, A. I.; Bowen, B. W.; Domning, D.; Mignucci-Giannoni, A. A.; Marmonel, M.; Montoya-Ospina, R.A. et al. (1998): Phylogeography of the West Indian manatee (Trichechus manatus). How many populations and how many taxa? In: *Mol Ecol* 7 (9), S. 1137–1149.

Gaspard, Joseph C. 3rd; Bauer, Gordon B.; Reep, Roger L.; Dziuk, Kimberly; Read, Latoshia; Mann, David A. (2013): Detection of hydrodynamic stimuli by the Florida manatee (Trichechus manatus latirostris). In: *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* 199 (6), S. 441–450.

Geraci, J.R., St. Aubin, D.J., and Hicks, B.D. (1986), The epidermis of odontocetes: A view from within, in Research on Dolphins, Bryden, M.M., and Harrison, R. (Eds.), Clarendon Press, Oxford, U.K., 3 - 21.

Gerstein, E. R. (Hg.) (1994): The manatee mind: Discrimination training for sensory perception testing of West Indian Manatees (Trichechus manatus). Marine Mammals: Puplic Display and Research (1).

Gerstein, Edmund R.; Gerstein, Laura; Forsythe, Steven E.; Blue, Joseph E. (1999): The underwater audiogram of the West Indian manatee (Trichechus manatus). In: *The Journal of the Acoustical Society of America* 105 (6), S. 3575–3583.

Gomes, Fabíola F. A.; Vergara-Parente, Jociery E.; Ferrari, Stephen F. (2008): Behaviour Patterns in Captive Manatees (Trichechus manatus manatus) at Itamaracá Island, Brazil - ProQuest. In: *Aquatic Mammals* 34 (3), S. 269–276.

Griebel, Ulrike; Schmid, Axel (1996): Color Vision in the Manatee (Trichechus manatus). In: *Vision Research* 36 (17), S. 2747–2757.

Harper, Jennifer Y. ; Schulte, Bruce A. (2005): Social interactions in captive female Florida manatees. In: Zoo Biol. 24 (2), S. 135–144.

Harrison, R.J. and King, J.E. (1965): Manatees and Dugongs. In: Marine Mammals, Hutchinson and Co LTD, London UK

Harrison, R.H., King, J.E. (1965): Marine mammals, second ed. Hutchinson, London

Hartman, D.S. (1971): Behaviour and ecology of the Florida manatee, *Trichechus manatus latirostris* (Harlan) at Crystal River, Citrus Country. Ph.D. Thesis (Zoology). Cornell University, 285 p.

Hartman, Daniel Stanwood (1979): Ecology and behavior of the manatee *(Trichechus manatus)* in Florida. Pittsburgh, USA: American Society of Mammalogists.

Hénaut, Yann; Becerra Lopez, Sylvia P.; Machkour-M'Rabet, Salima; Morales-Vela, Benjamín; Winterton, Peter; Delfour, Fabienne (2010): Activities and social interactions in captive Antillean manatees in Mexico. In: *mammalia* 74 (2).

Holguin-Medina, Victoria Eugenia; Fontenele-Araujo, John; Alcaraz-Romero, Víctor Manuel; Cortes, Jose Francisco; Muñoz-Delgado, Jairo (2015): Circadian and ultradian activity rhythms in manatee (Trichechus manatus manatus) in captivity. In: *Biological Rhythm Research* 46 (5), S. 631–645.

Horikoshi-Beckett, Chifuyu; Schulte, Bruce A. (2006): Activity patterns and spatial use of facility by a group of captive female manatees (Trichechus manatus latirostris). In: *Zoo Biol.* 25 (4), S. 285–301.

Husar, Sandra L. (1977): The West Indian manatee (Trichechus manatus). In: *Wildlife Research Report* (7).

Husar, S.L. (1977b): *Trichechus inunguis*. Mamm. Species 72: 1 - 4

Irvine, A.B., Neal, R.C., Cardeilhac, R.T., Popp, J.A., Whiter, F.H., Jenkins, R.C. (1980): Clinical observations on captive and free-ranging West Indian manatees, Trichechus manatus. In: Aquatic Mammals 8, S. 2 - 10

Keaffaber, J.J., Coston, C.J., Ham, W.G., Burns, T., Sherrill, A., Khan, S. (1992) Redox chemistry of closed ozonated systems: Modeling bromine and other redox active species. in: Blogoslawski W.J. (ed.), Proceedings of the Third International Symposium on the Use of Ozone in Aquatic Systems. Pan American Group of the International Ozone Association, Stamford, CT, pp. 76/91.

Ketten, Darlene R.; Odell, Daniel K.; Domning, Daryl P. (1992): Structure, Function, and Adaptation of the Manatee Ear. In: Jeanette A. Thomas, Ronald A. Kastelein und A. I☐A☐ Supin (Hg.): Marine Mammal Sensory Systems. Boston, MA: Springer US, S. 77–95.

Larkin, I.V., Gross, T.S., and Reep, R.L. (2005): Use of Faecal Testosterone Concentrations to Monitor Male Florida Manatee *(Trichechus manatus latirostris)* Reproductive Status. Aquatic Mammals 31(1): 52- 61

Marmontel, Miriam; Nhea, Thomas J.; Kochman, Howard I.; Humphrey, Stephen R. (1996): Age determination in manatees using growth-layer-group counts in bone. In: *Marine Mammal Sci* 12 (1), S. 54–88.

Marmontel, M. (1988): The Reproductive Anatomy of the Females Manatee Trichechus manatus latirostris (Linnaeus 1758) Based on Gross and Histologic Observations. Master thesis, University of Miami

Mass, A. M.; Odell, D. K.; Ketten, D. R.; Supin, A. Ya. (1997): Ganglion Layer Topography and Retinal Resoulution of the Caribbean Manatee Trichechus manatus latirostris. In: *Doklady Biological Sciences* 355, S. 392–394.

Mass, Alla M.; Supin, Alexander Ya (2007): Adaptive features of aquatic mammals' eye. In: *Anatomical record (Hoboken, N.J. : 2007)* 290 (6), S. 701–715.

Montgomery, G. Gene; Best, Robin C.; Yamakoshi, Megumi (1981): A Radio-Tracking Study of the Amazonian Manatee Trichechus inuguis (Mammalia. Sirenia). In: *Biotropica* 13 (2), S. 81.

Moore, Joseph Curtis (1957): Newborn Young of a Captive Manatee. In: *Journal of Mammalogy* 38 (1), S. 137.

Nowacek, Douglas P.; Casper, Brandon M.; Wells, Randall S.; Nowacek, Stephanie M.; Mann, David A. (2003): Intraspecific and geographic variation of West Indian manatee (Trichechus manatus spp.) vocalizations (L). In: *J. Acoust. Soc. Am.* 114 (1), S. 66.

Odell, G.D., Bossart, M.T., Lowe, and T.D. Hopkins (1995): Reproduction of the West Indian manatee in captivity. Abstract. Pp 192 - 193 in T.J. O'Shea, B.B. Ackerman, and H.F. Percival (eds.), *Population biology of the Florida manatee*. National Biological Service Information and Technical Report 1. 289 pp.

Ortiz, R. M.; Worthy, G. A.; Byers, F. M. (1999): Estimation of water turnover rates of captive West Indian manatees (Trichechus manatus) held in fresh and salt water. In: *Journal of Experimental Biology* 202, S. 33–38.

Rathbun, G. B.; Reid, J. P.; Bonde, R. K.; Powell, J. A. (1995): Reproduction in free-ranging Florida manatees (Trichechus manatus latirostris). In: *Information and Technology Report* (1).

Reep, R. L.; Bonde, R. K. (2006): The Florida Manatee: Biology and Conservation

Reep, R. L.; Stoll, M. L.; Marshall, C. D.; Homer, B. L.; Samuelson, D. A. (2001): Microanatomy of Facial Vibrissae in the Florida Manatee. The Basis for Specialized Sensory Function and Oripulation. In: *Brain Behav Evol* 58 (1), S. 1–14.

Reid, J. P., Bonde, R.K. and T.J. O'Shea. (1995): Reproduction and mortality of radio tagged and recognizable manatees on the Atlantic coast of Florida. Pp. 171 - 191 in T.J. O'Shea, B.B. Ackerman, and H.F. Percival (eds.), Population biology of the Florida manatee. National Biological Service Information and Technical Report 1. 289 pp.

Reynolds, J.E. (1977): Aspects of the social behavior and ecology of a semi-isolated colony of Florida manatees, Trichechus manatus. M.Sc. thesis, University of Miami, Coral Gables, Florida

Reynolds J.E. (1981): Behavior patterns in the west indian manatee, with emphasis on feeding and diving [Trichechus manatus, Florida]. In: *Florida Science*.

Reynolds, J.E., III (1980): Aspects of the structural and functional anatomy of the gastrointestinal tract of the West Indian manatee, *Trichechus manatus.* Ph.D. Thesis, University of Miami, Coral Gables, Florida

Reynolds, J.E. III, and D.K. Odell (1991): Manatees and Dugongs. Facts on File, Inc., New York. ISBN 0-8160-2436-7: 192 pp

Reynolds, J. E., III, S. A. Rommel and M. E. Pitchford. (2004): The likelihood of sperm competition in manatees - explaining an apparent paradox. Marine Mammal Science 20: 464-476.

Ridgway & R.J. Harrison (Eds). Handbook of marine mammals. London, Academic Press, Vol. 3, 362p.

Rommel, Sentiel; Reynolds J.E. (2000): Diaphragm structure and function in the Florida manatee (Trichechus manatus latirostris). In: *The Anatomical Record* 259 (1).

Scholander, P.F. & Irving, L. (1941): Experimental investigations on the respiration and diving of the Florida manatee. In: *J. Cell. Comp. Physiol*. 17, 169 - 191

Sousa-Lima, Renata S.; Paglia, Adriano P.; Fonseca, Gustavo A B da (2008): Gender, Age, and Identity in the Isolation Calls of Antillean Manatees (Trichechus manatus manatus) - ProQuest. In: *Aquatic Mammals* 34 (1), 109-112,114-115,117-122.

Summerfelt, S.T., Hochheimer, J.N. (1997) Review of ozone processes and applications as an oxidizing agent in aquaculture. The Progressive Fish-Culturist 59, 94/105.

Vianna, Juliana A.; Bonde, Robert K.; Caballero, Susana; Giraldo, Juan Pablo; Lima, Regis P.; Clark, Annmarie et al. (2006): Phylogeography, phylogeny and hybridization in trichechid sirenians: implications for manatee conservation. In: *Molecular ecology* 15 (2), S. 433–447.

Walsh, M. T., and G. D. Bossart. (1999): Manatee medicine. In: Zoo and Wildlife Medicine, M. E. Fowler and R. E. Miller (eds.). W. B. Saunders, Philadelphia, Pennsylvania, pp. 507–516.

Wells, R. S., D. J. Boness and G. B. Rathbun (1999): Behavior. Pages 324 - 422 in J. E. Reynolds, III and S. A. Rommel eds. Biology of Marine Mammals. Smithsonian Institution Press, Washington, D. C.

White, J. R., Harkness, D. R. , Isaaks, R. E. , and Duffield, D. A. (1976): Some studies on blood of the Florida manatee, *Trichechus manatus latirostris*. In: *Comp. Biochem. Physiol.* 55: 413

White, J.R. and R. Francis-Floyd (1990): Manatee biology and medicine. Pp. 601 - 623 in L.A. Dierauf, ed. C*RC Handbook of marine mammal medicine: Health, disease and rehabilitation*. CRC Press, Boca Raton, FL

Yamasaki, F., S. Komatsu, and T. Kamiya (1980): A comparative morphological study on the tongues of manatee and dugong (Sirenia). In: Scientific Reports of the Whales Research Institute (Tokyo) 32: 127 - 144.